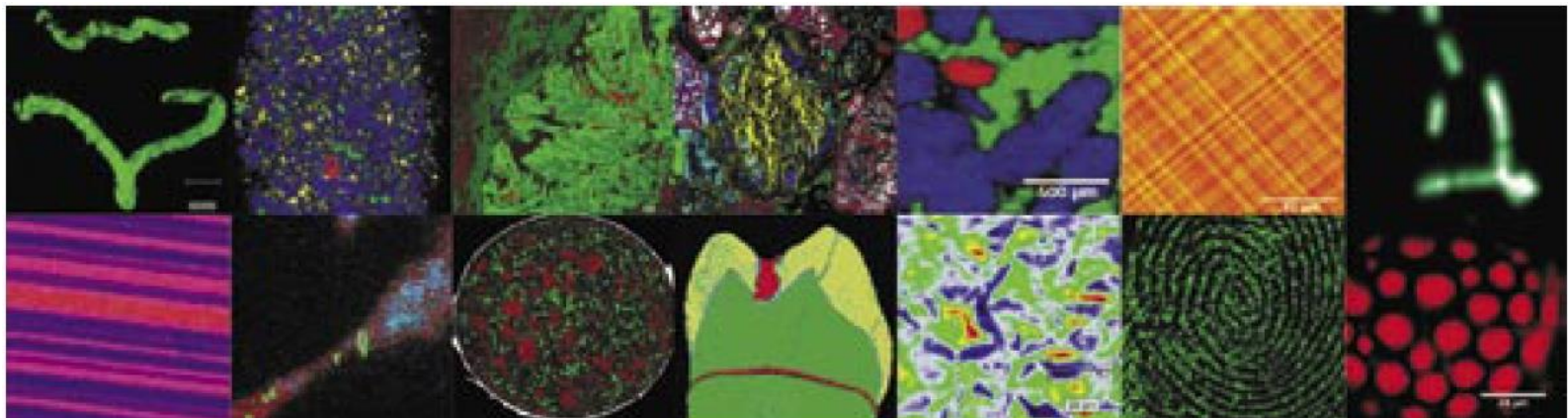


Raman effect and CARS

History, a little bit of theory and applications

Dr. Micha Kölbach

Abteilungsleiter Spektroskopie, Renishaw GmbH



Outlook

- History
- Raman-Effect / Theory
- Setup
- Applications in biology
- CARS basics

Slides marked with * are from RAMAN-SPEKTROSKOPIE
VORTRAG IM RAHMEN DER VORLESUNG ANWENDUNGEN UND METHODEN DER MINERALOGIE
Prof. Dr. Volker Presser

History – Raman-Effect

- Predicted by physicist Adolf Smekal in 1923
- discovered and named by Sir Chandrasekhara Venkata Raman in 1928
- Nobelprice for Sir C.V. Raman in 1930



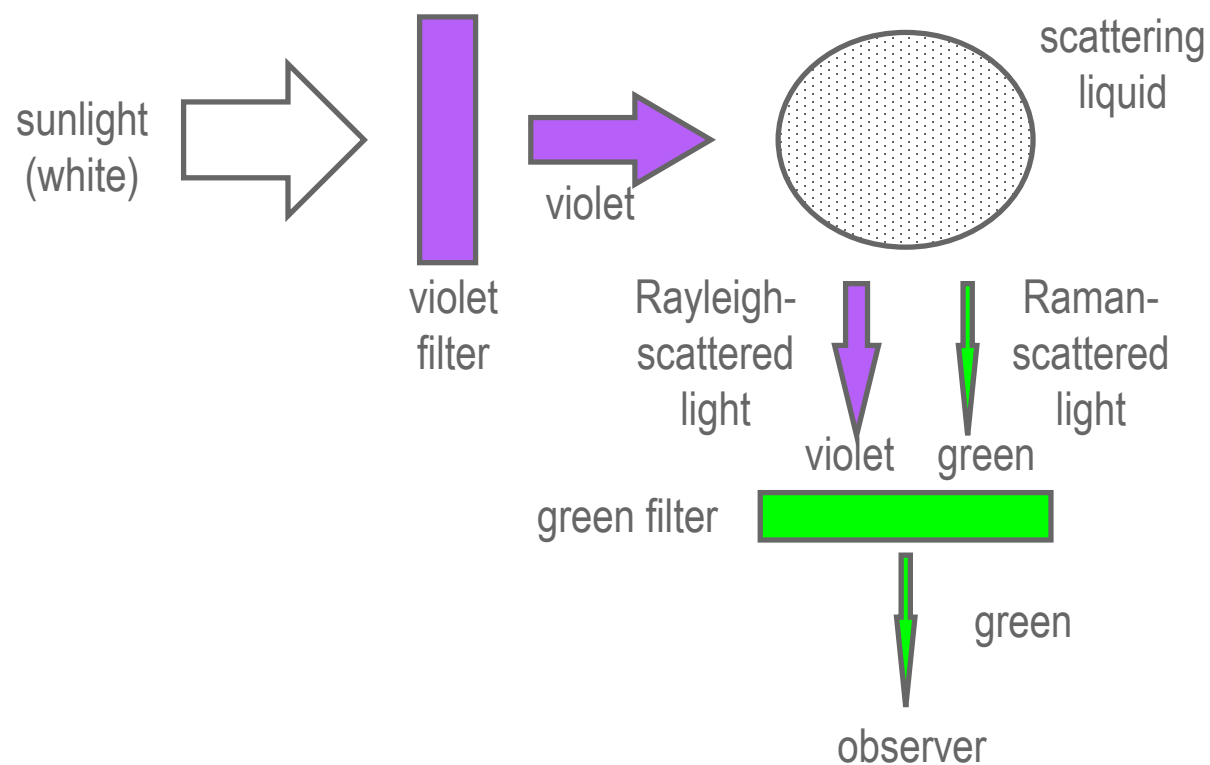
Sir C. V. Raman

History – Raman-Effect

- inelastical scattering of sun light at liquids
- used simple filter technique for the experiment
- Lansberg and Mandelstam showed the effect almost at same time with quartz crystals



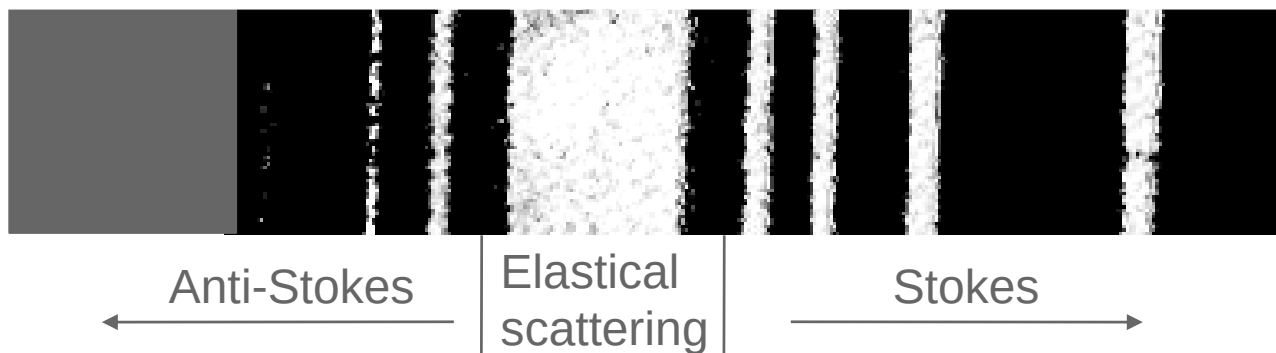
Raman's experiment



What does Raman effect describes?

*

- Laser light illuminates the sample (solid state, liquids or gases)
- Most of the light scatters off without change in energy (elastic)
- A tiny fraction is inelastically scattered, i.e. Raman scattered
- This change in energy is specific to the vibrational (or rotational) modes of the molecules
- The spectrum of this scattered light allows us to identify the sample and better understand its composition
- **Example:** CCl_4 -spectrum (C. V. Raman, 1929; 40 h integration time!)



Classical theory of Raman-Effect

- The electric field E_i of the light wave acts on the charges in the material
- Interaction of light with a single molecule considered
- Induced dipole moment P_i of a molecule (vector)

$$P_i = \mu_i + \alpha_{ij}E_j + \beta_{ijk}E_jE_k \quad (1)$$

μ_i – induced permanent dipole moment

α_{ij} – polarizability (tensor)

i, j, k – subscripts running over directions x, y, z

Classical theory of Raman-Effect

Both μ_i and α_{ij} may change if the molecule vibrates

Then μ_i and α_{ij} may be expanded as Taylor series

$$\mu_i = \mu_{0i} + \sum_n \left(\frac{\partial \mu_i}{\partial q_n} \right)_{q=0} q_n$$

$$\alpha_{ij} = \alpha_{0ij} + \sum_n \left(\frac{\partial \alpha_{ij}}{\partial q_n} \right)_{q=0} q_n$$

q_n – generalized co-ordinates of normal modes

Assuming small atomic displacements q_n , we can approximate the time dependence:

$$q_n(t) = q_{n0} \cos(\omega_n t)$$

And for the E-Field of the incident light: :

$$E_i(t) = E_{0i} \cos(\omega_L t)$$

ω_n, ω_L – frequency of displacement and electric field

These expressions can be substituted into a linear version of (1)

Classical theory of Raman-Effect

$$\bullet P_i = \mu_i + \alpha_{ij}E_j + \beta_{ijk}E_jE_k = P_i = \mu_i + \alpha_{ij}E_j + K$$

$$= \mu_{0i} + \sum_n \left(\frac{\partial \mu_i}{\partial q_n} \right)_{q=0} q_{n0} \cos(\omega_n t) + \alpha_{0ij} E_{0i} \cos(\omega_L t) \\ + \sum_n \left(\frac{\partial \alpha_{ij}}{\partial q_n} \right)_{q=0} q_{n0} \cos(\omega_n t) E_{0i} \cos(\omega_L t) + K$$

Rayleigh-Scattering

$$= \mu_{0i} + \sum_n \left(\frac{\partial \mu_i}{\partial q_n} \right)_{q=0} q_{n0} \cos(\omega_n t) + \alpha_{0ij} E_{0i} \cos(\omega_L t)$$

IR-absorption

$$+ q_{n0} E_{0i} \frac{1}{2} \sum_n \left(\frac{\partial \alpha_{ij}}{\partial q_n} \right)_{q=0} \{ \cos(\omega_L + \omega_n) t + \cos(\omega_L - \omega_n) t \} + K$$

Raman-Scattering with Stokes and Antistokes

Classical theory of Raman-Effect

*

Polarizability and dipole moment (1)

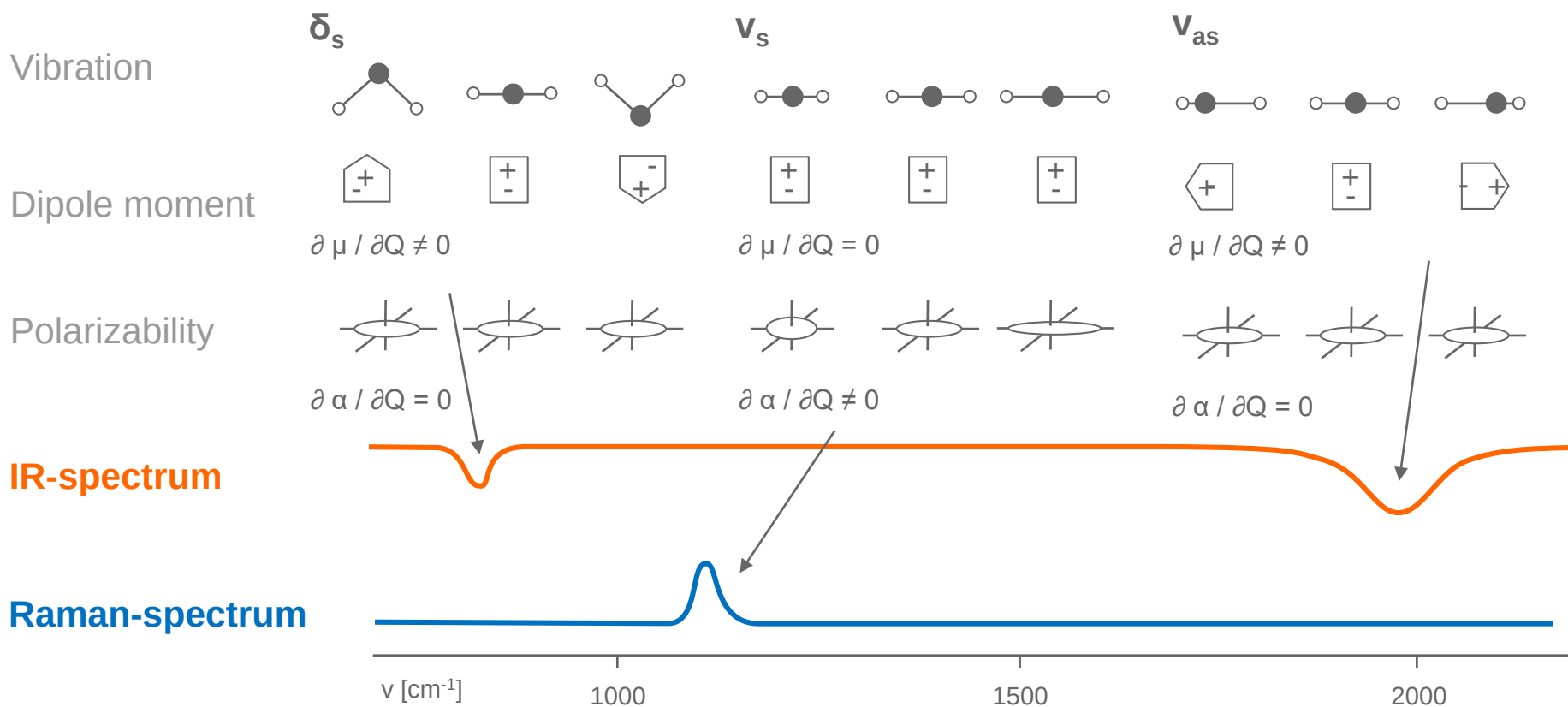
- IR-activity = change of the dipole moment
→ While vibration center of mass of electron cloud changes
 - Raman-activity = change of polarizability
→ During vibration shape of electron cloud changes
- What does this mean?

Classical theory of Raman-Effect

*

Polarizability and dipole moment (2)

- Simple example: CS_2



Theory of Raman – Quantum mechanics

Raman effect classically explained!

- IR
- Rayleigh
- Raman
 - Stokes
 - Antistokes

However: Why are there so extrem differences in intensity of Antistokes and Stokes?

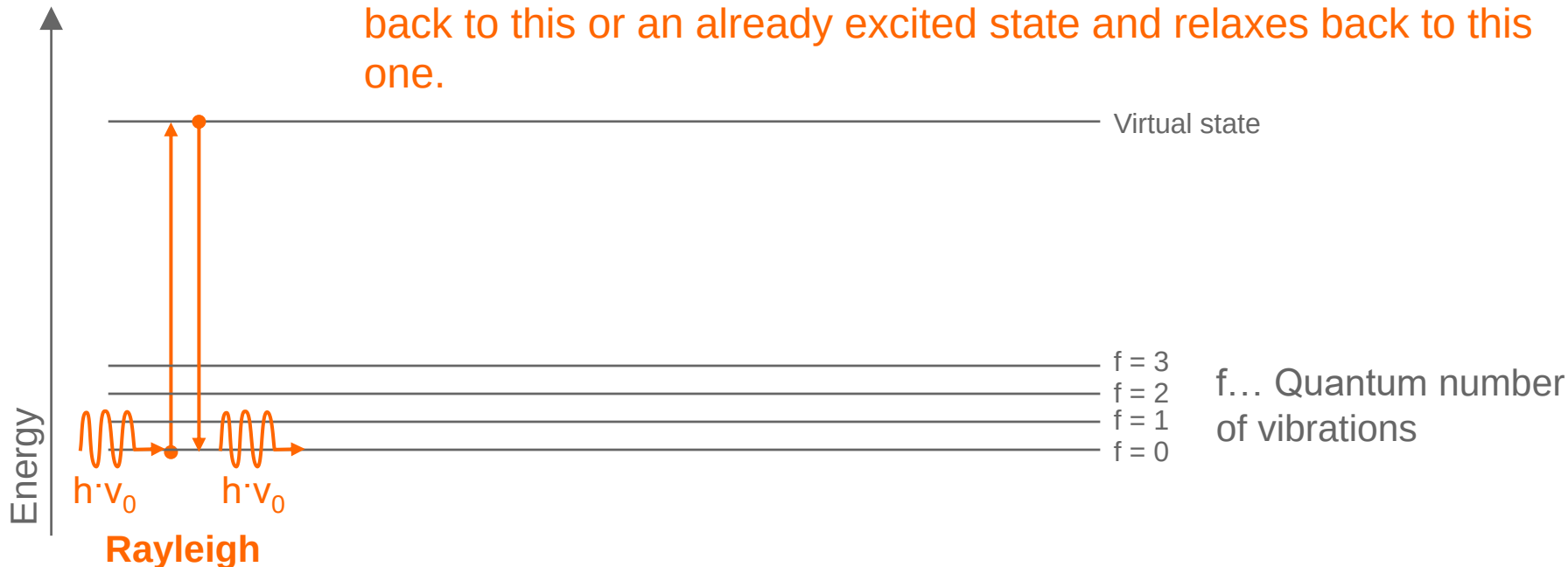
Theory of Raman – Quantum mechanics

*

Rayleigh, Stokes, Anti-Stokes and a little bit of quantum mechanics

- **Case 1: Rayleigh Scattering**

- the molecule is lifted in an excited virtual state of vibration and relaxes back into ground state. The emitted/scattered light has the same energy as the excitation wavelength
- it doesn't matter whether it starts from ground state and goes back to this or an already excited state and relaxes back to this one.



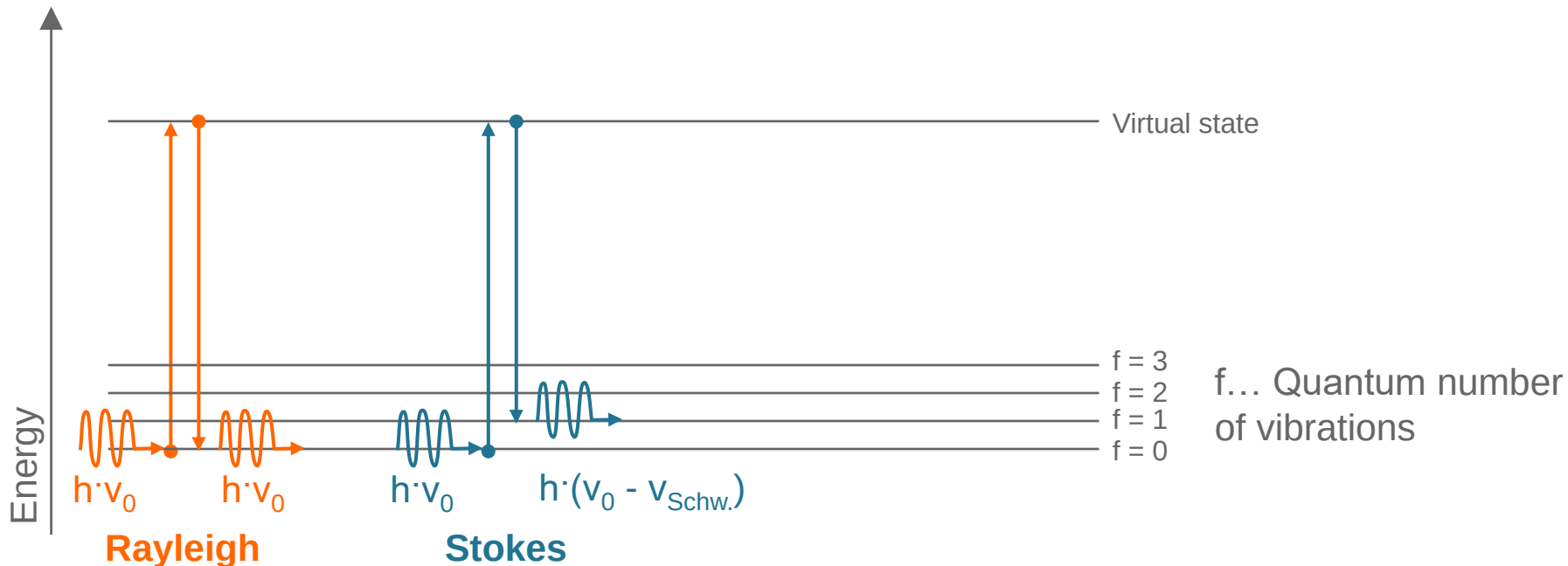
Theory of Raman – Quantum mechanics

*

Rayleigh, Stokes, Anti-Stokes and a little bit of quantum mechanics

- **Case 2: Stokes**

→ Stokes Raman scattering leads the molecule into an excited vibrational state after relaxation from virtual state. The emitted light has a lower energy than the excitation wavelength has



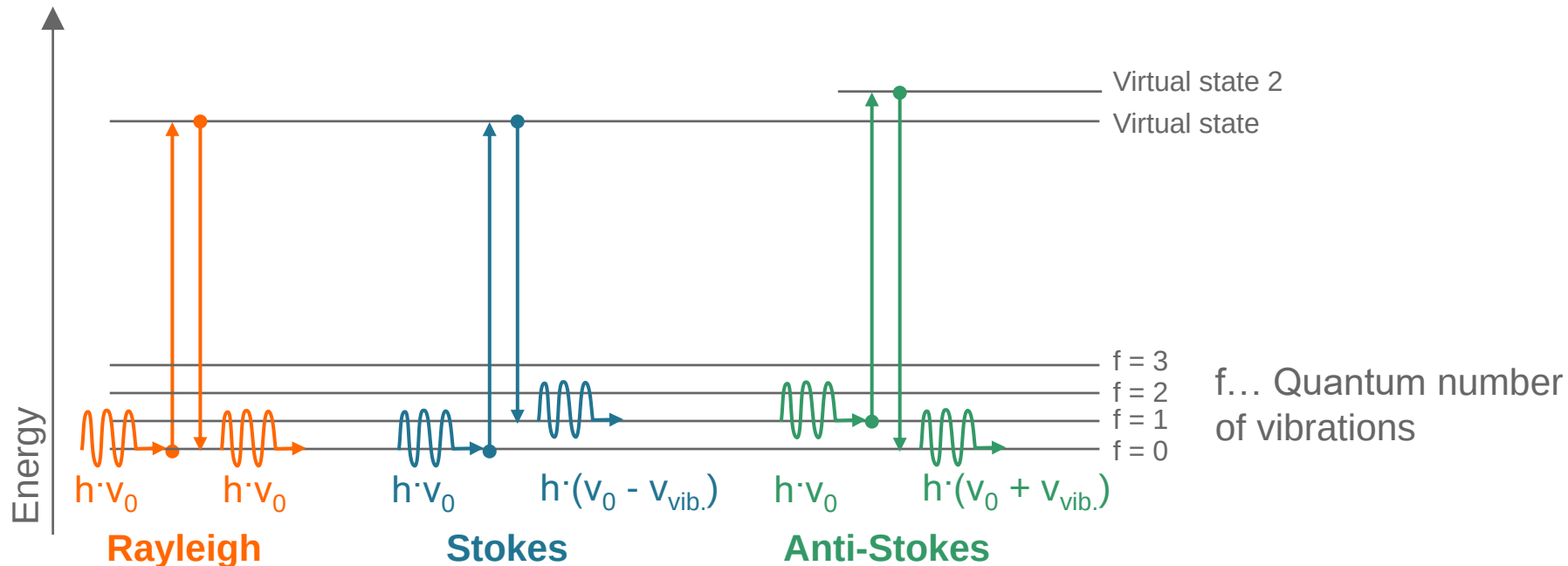
Theory of Raman – Quantum mechanics

*

Rayleigh, Stokes, Anti-Stokes and a little bit of quantum mechanics

- **Case 3: Anti-Stokes**

→ Anti-Stokes-Raman scattering leads to a molecule in ground state starting from an excited vibrational state. The emitted light has a higher energy than the excitation wavelength has



Theory of Raman – Quantum mechanics

*

Stokes, Anti-Stokes and the question about intensity of peaks

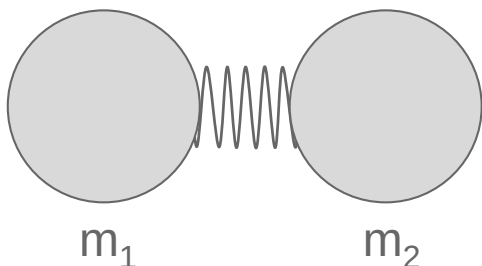
- In principle: inelastic scattering is not standard!
Only 1 of around 10 millionen photons is inelastically scattered
Slightly comparable with firefly one wants to see in full sun light.
 - Rayleigh peak is orders of magnitudes stronger than any Raman peak!
- Anti-Stokes-scattering needs excited vibrational states
 - At room temperature only small number of molecules are excited (calculation with Boltzmann-distribution leads to around 0,04%). Therefore Stokes peaks are usually much stronger than Anti Stokes peaks.

Theory of Raman

*

Position of Raman peaks (1)

- Position of Raman peaks can be described in a classical approach by a harmonical (or anharmonical) oscillator:
 - Two atoms are connected by a spring = covalent bond
 - Frequency of stretch vibration ν can therefore be calculated by using the reduced mass m^* spring rate k



$$m^* = \frac{m_1 \cdot m_2}{m_1 + m_2} \quad \nu = \frac{1}{2\pi} \cdot \sqrt{\frac{k}{m^*}}$$

Theory of Raman

*

Position of Raman peaks (2)

- With this formular is described...
 - ... why for weaker bonds the vibration modes are located at lower frequencies
 - ... why vibrations of more heavy atoms are at lower frequencies (the higher the difference in mass between the atoms, the higher the frequency shift, for example D vs. H)

Theory of Raman

*

What is usually plotted in a Raman spectrum?

- Intensity vs. Frequency is not that useful since the spectrum depends in that case on the excitation wavelength.
- Better: use of Raman shift instead, i.e. difference between Raman peak and Rayleigh peak in wavenumber. Then the spectrum is independent of excitation wavelength
- What is „+“ and what is „-“?

– *Definition:* $\Delta\nu = \nu_0 - \nu_{\text{Peak}}$ & $\nu_{\text{Peak}} = \nu_0 \pm \nu_{\text{vibration}}$

→ Anti-Stokes = higher energy:

$\nu_0 < \nu_{\text{Peak}}$, d.h. $\Delta\nu < 0$ MINUS

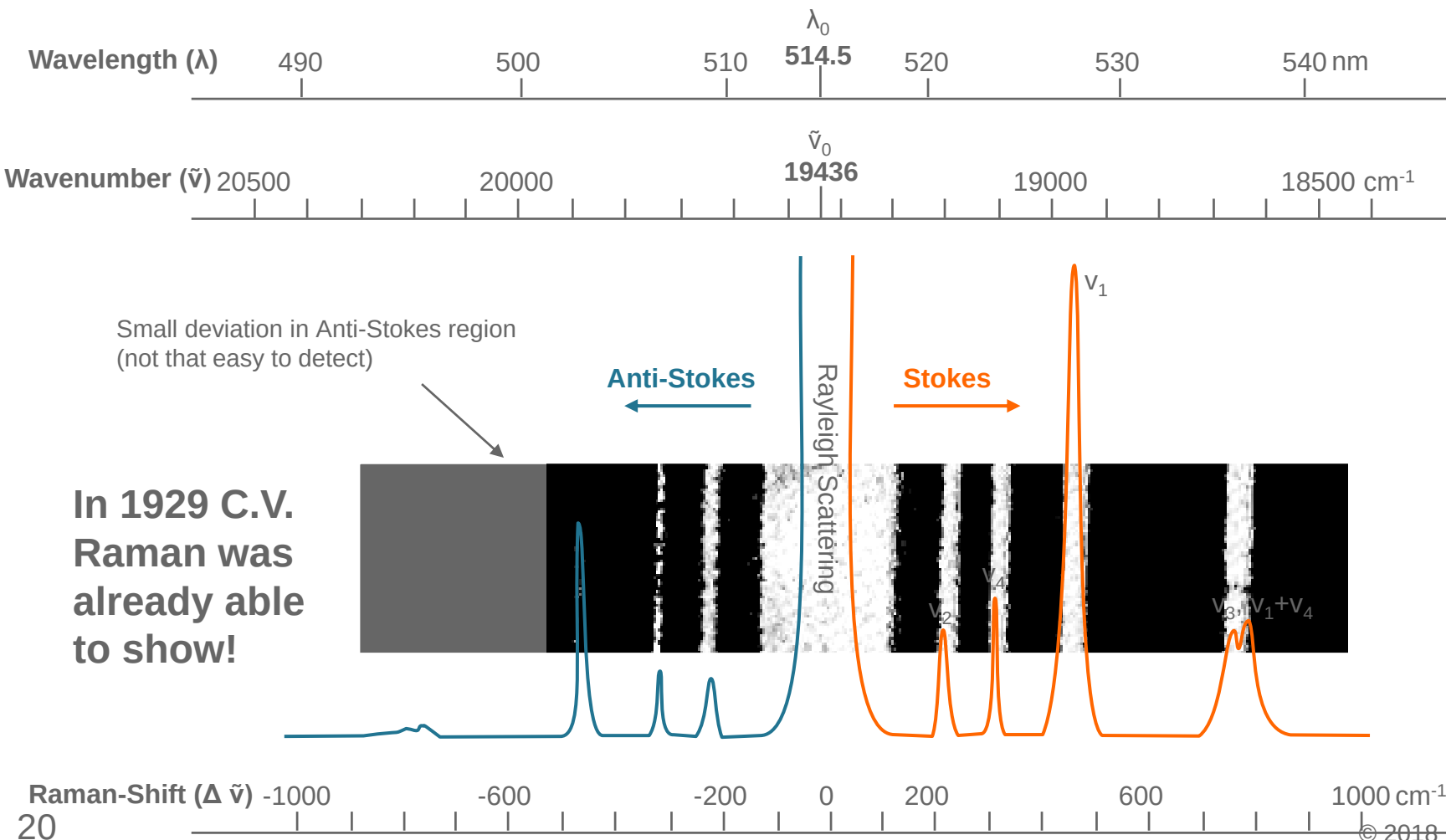
→ Stokes = lower energy:

$\nu_0 > \nu_{\text{Peak}}$, d.h. $\Delta\nu > 0$ PLUS

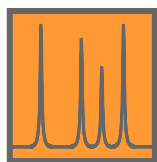
Theory of Raman

*

Example of a Raman spectrum (514.5 nm, CCl₄)



Information by Raman



characteristic
Raman frequencies

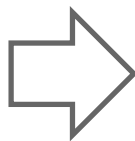


composition of
material

e.g. MoS₂, MoO₃

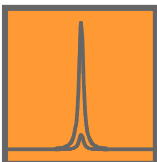


changes in
frequency of
Raman peak



stress/strain
state

e.g. Si 10 cm⁻¹ shift per
% strain

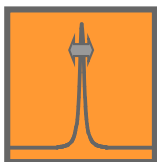


polarisation of
Raman peak



crystal symmetry and
orientation

e.g. orientation of CVD
diamond grains

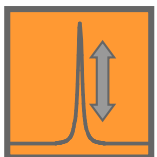


width of Raman
peak



quality of crystal

e.g. amount of plastic
deformation



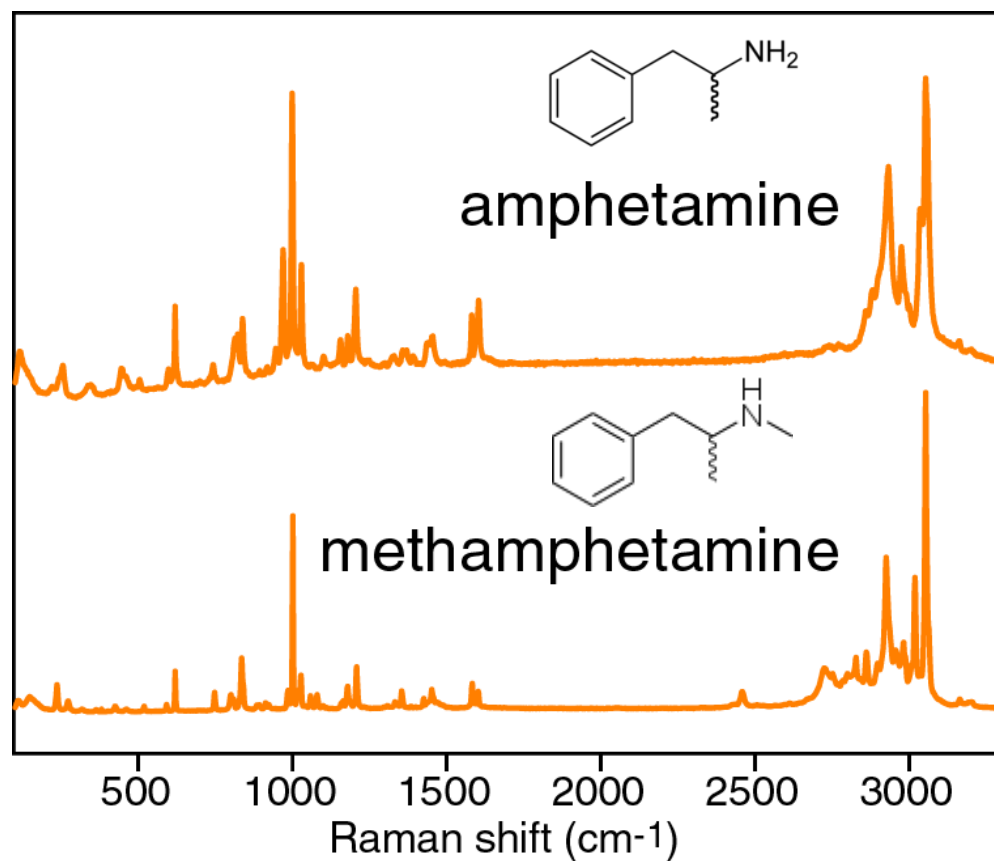
intensity of
Raman peak



amount of material

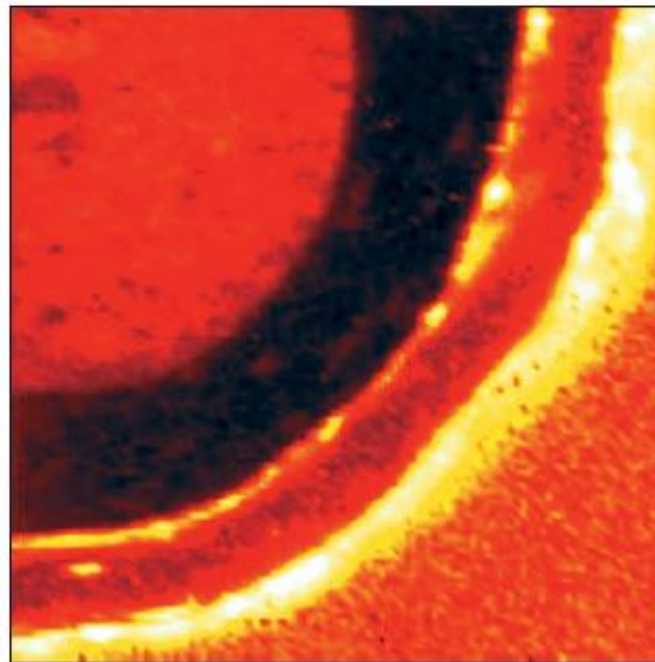
e.g. thickness of
transparent coating

Example



Advantages of Raman spectroscopy

- Non-destructive
- Sub micrometre spatial resolution
 - ~300 nm in visible
- Flexible sampling
 - any phase
 - any size
 - no preparation
- Glass/water - transparent
 - bio samples
 - fibre optics
 - inside reactors
- Fast chemical imaging

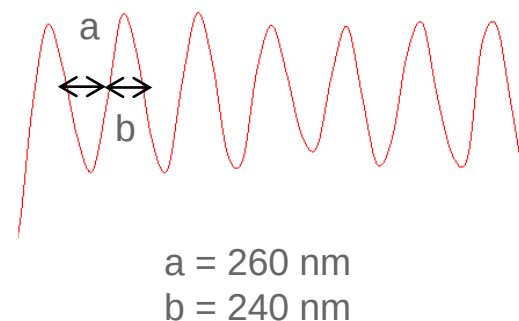
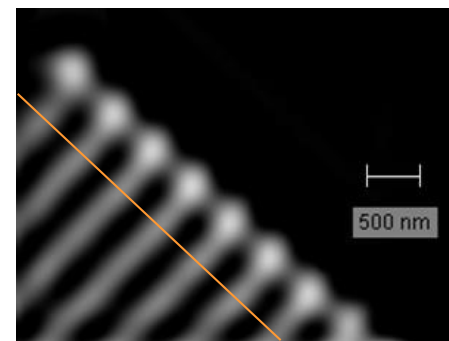


**Line focus image showing
layers in a pharmaceutical
bead formulation (image area
500 µm x 500 µm)**

Acknowledgement:
R. Brody and D. Clark,
Pfizer Global Research and Development, Sandwich, UK

Limitations of Raman: spatial resolution

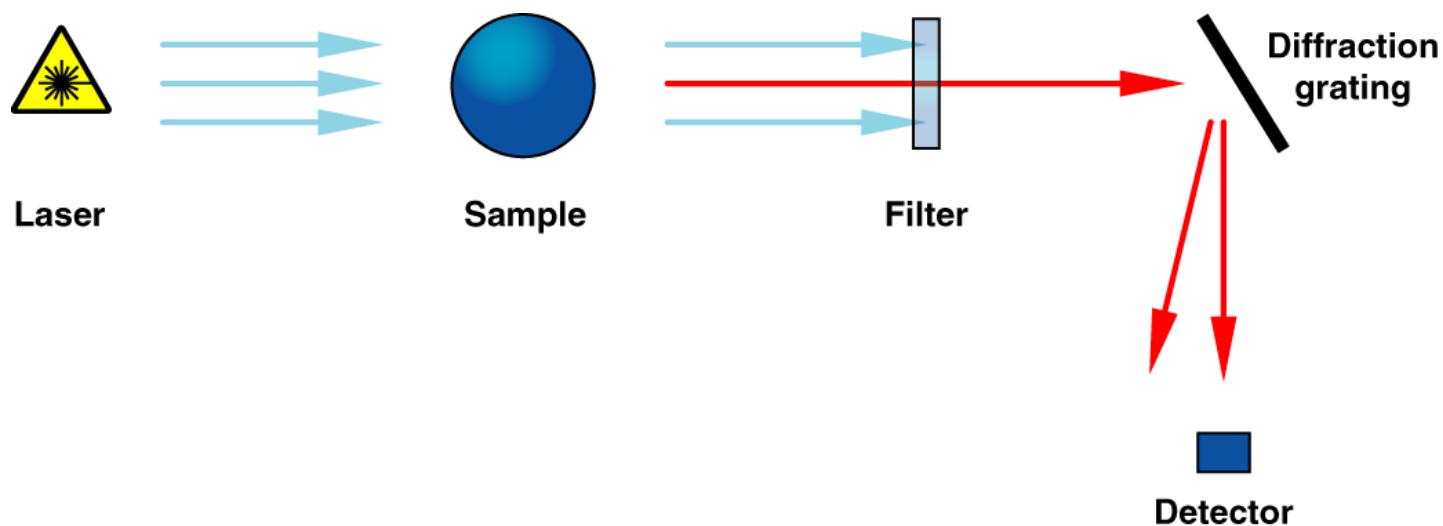
- Spatial resolution limited by wavelength of light
- Rayleigh resolution (R_{xy})
 - $(R_{xy}) = 0.61 \lambda / NA$
where λ is laser wavelength and
NA is the numerical aperture of the lens
- Best spatial resolution ~250 nm
- Great for most things but a problem for
 - Latest electronic devices
 - Nanotechnology
 - Cell biology



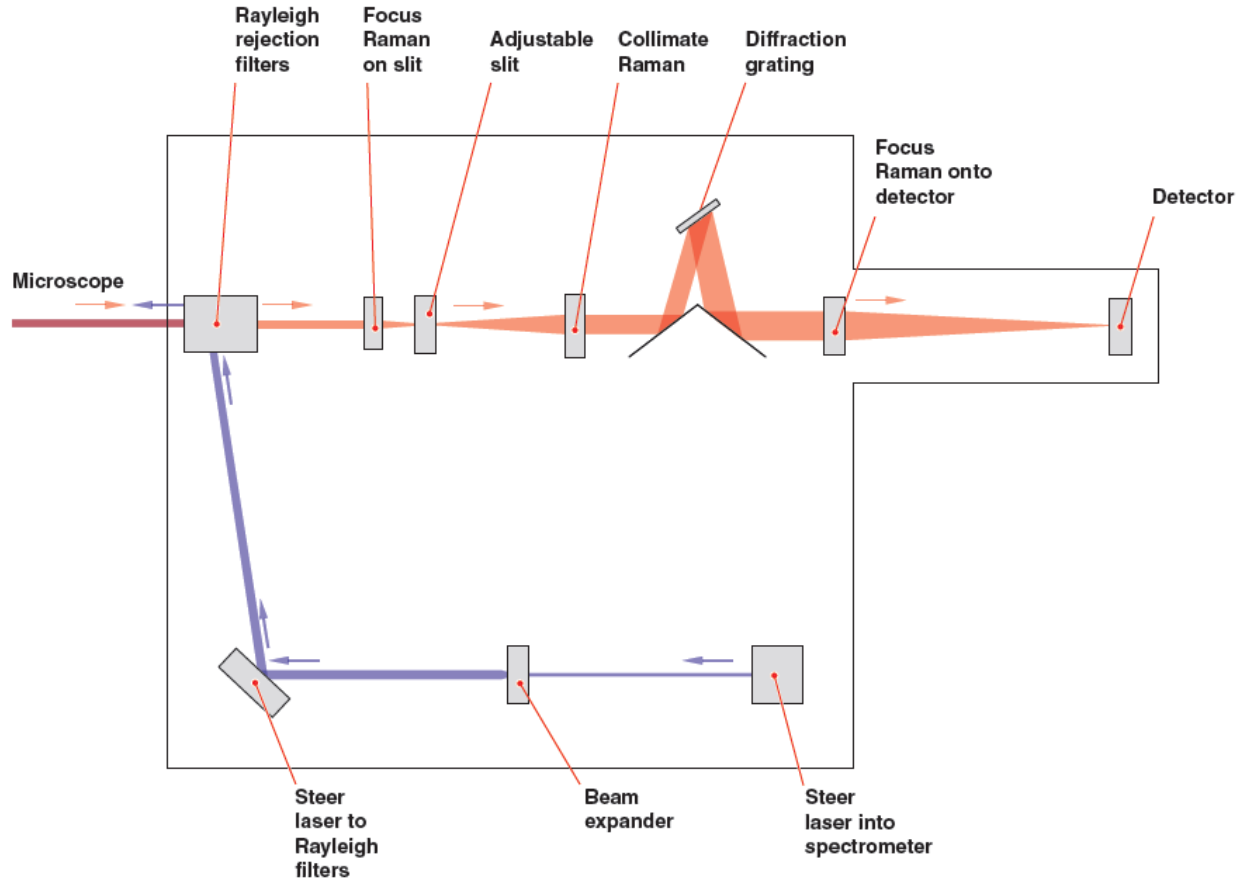
**Solution! Combined Raman-AFM
TERS resolutions of < 20 nm**

Basic setup of a Raman experiment

- Excitation with monochromatical light (Laser)
- blocking of elastically scattered light (Rayleigh) by use of filter or double pre monochromator
- dispersive element (grating)
- Detection of inelastically scattered light (Raman)



Setup of spectrometer – lens based



Research grade microscope
Confocal operation
Motorised beam steers

Holographic notch filters
Dielectric edge filters
Motorised gratings

Low noise CCD detector
Extended scanning range
Multiple lasers

Strength and weakness of lens based spectrometer

•Strength :

On-Axis Setup: the light path is always along the optical axis

→no Astigmatism

→no Spherical Abberation

→even short focal length lead to high spectral resolution

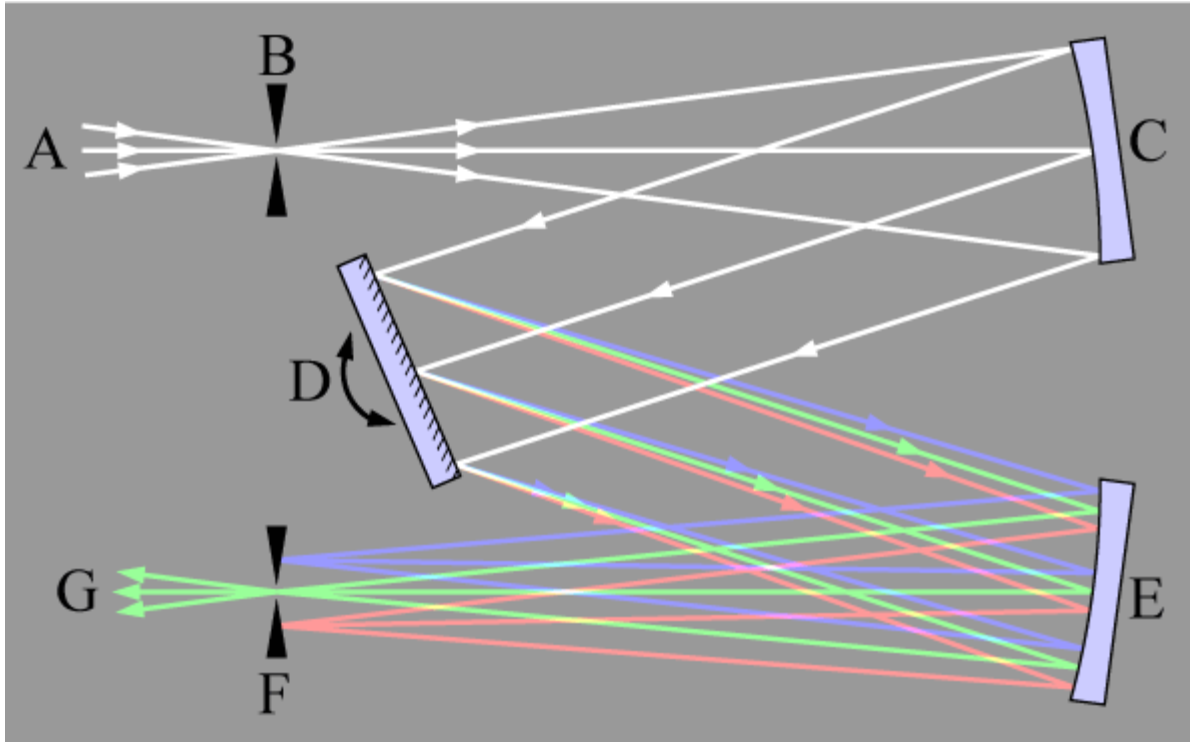
→therefore smaller optics (lenses, gratings) get very high light throughput

•Weakness:

chromatical Abberation

BUT: this can be solved by optimised optics

Czerny-Turner Spectrometer



<https://de.wikipedia.org/wiki/Monochromator#/media/File:Czerny-turner.png>

Von Der ursprünglich hochladende Benutzer war DrBob in der Wikipedia auf Englisch - Übertragen aus en.wikipedia nach Commons durch Leyo mithilfe des CommonsHelper., CC BY-SA 3.0, <https://commons.wikimedia.org/w/index.php?curid=9934696>

Strength and weakness of Czerny-Turner Spectrometer

- **Strength:**

No chromatical Abberation

Well known design

Multiple Stage-Setup possible

- **Weakness**

Off-Axis Design: light is not on optical axis

→ Astigmatism

→ Spherical Abberation

→ High focal length neccesary to achieve high spectral resolution

→ to obtain high light troughput it is neccessary to use big and therefore expensive optics

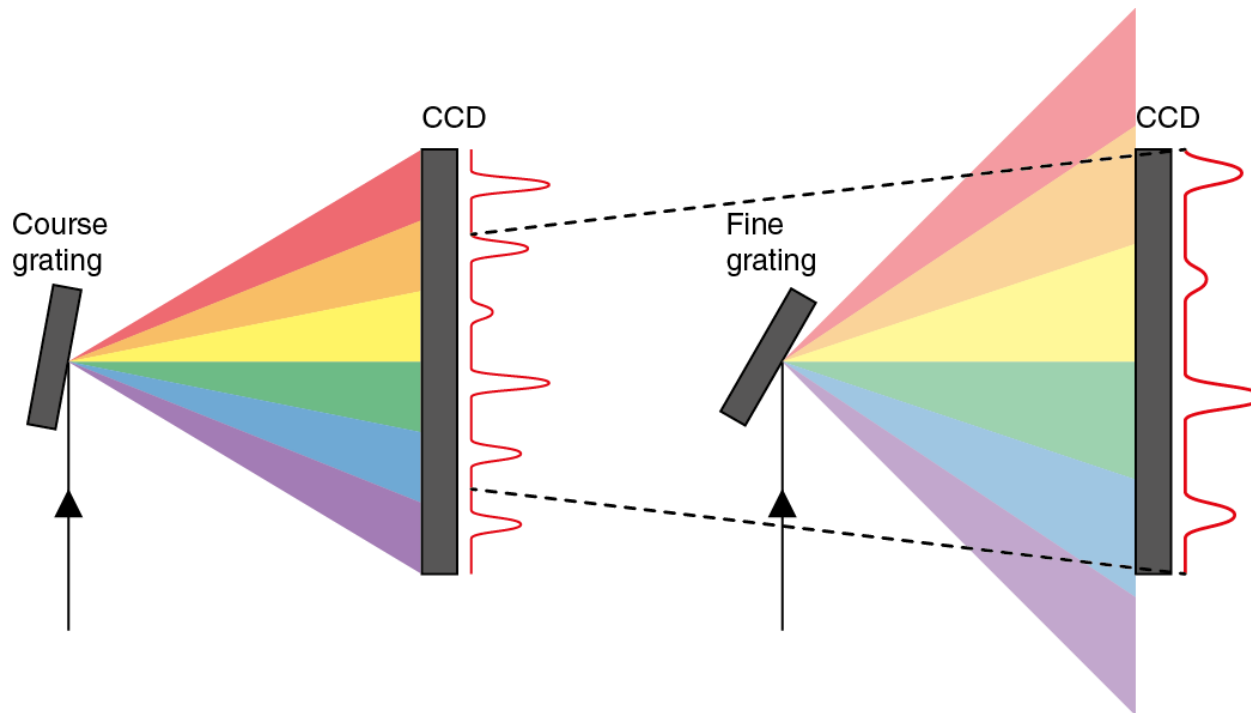
Gratings

Gratings are the **dispersive element**:

- Grating acts similar to a prism,
- Grating disperses the Raman light
- discrete lines / peaks on CCD
- there is a big variety of different gratings available from 150 l/mm up to 4000 l/mm

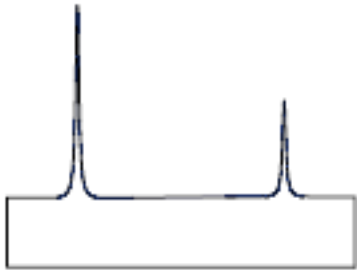
Grating

The higher the l/mm the higher the spectral resolution,
BUT also: the smaller the spectral range on the CCD
AND the lower the intensity per pixel

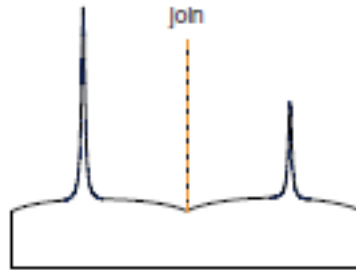


SynchroScan

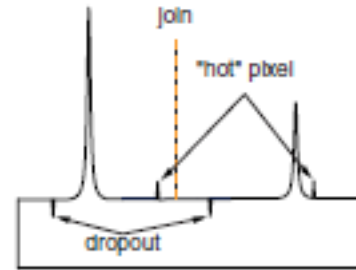
- Extending scan range
- Normal procedure – “stitching” of spectra



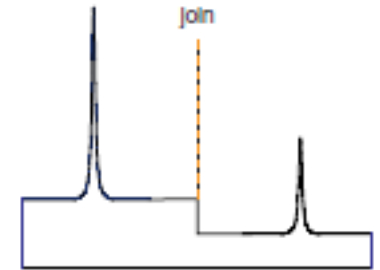
True spectrum



Optical aberrations
artefacts

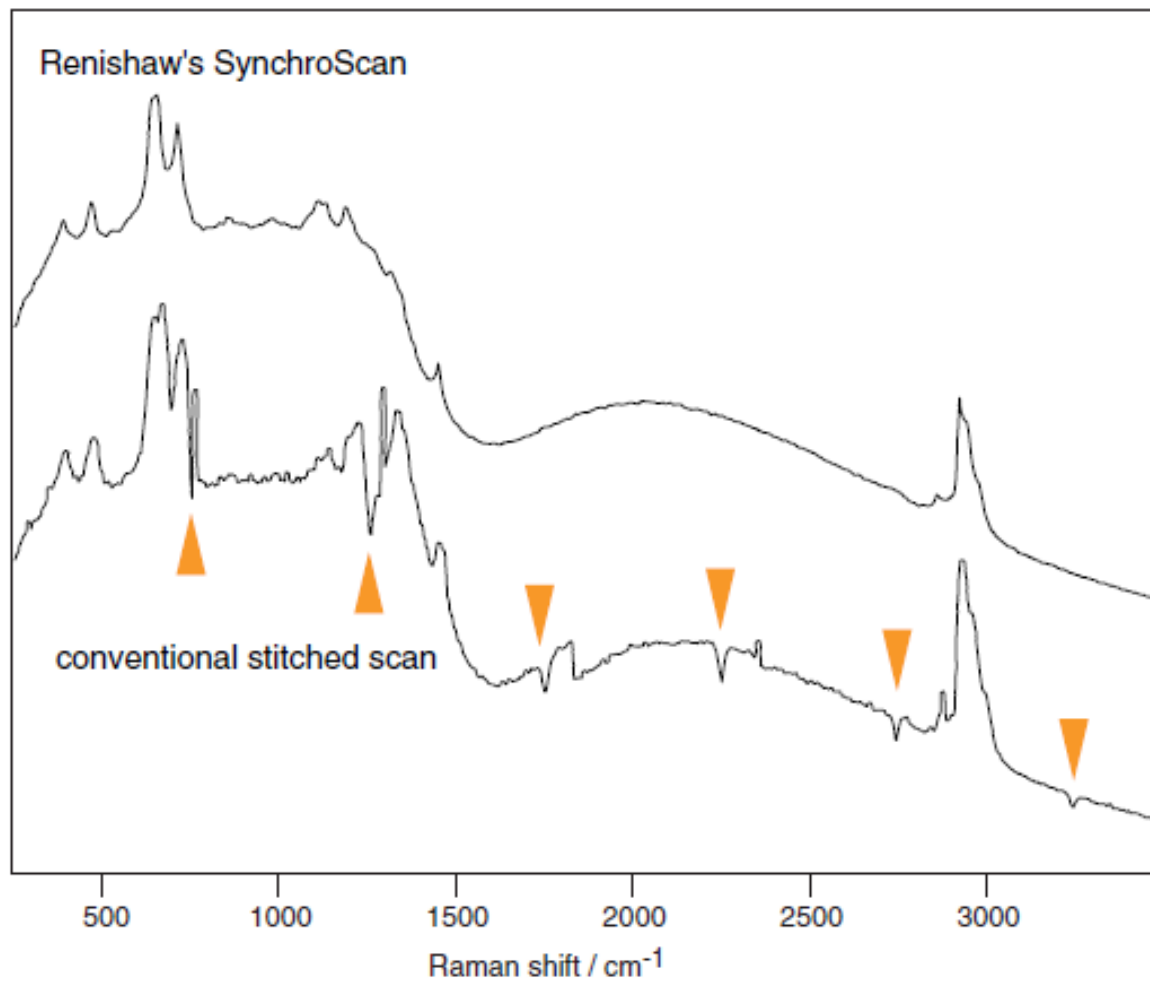


„hot pixel“ and dropout
artefacts
(CCD charge leakage)



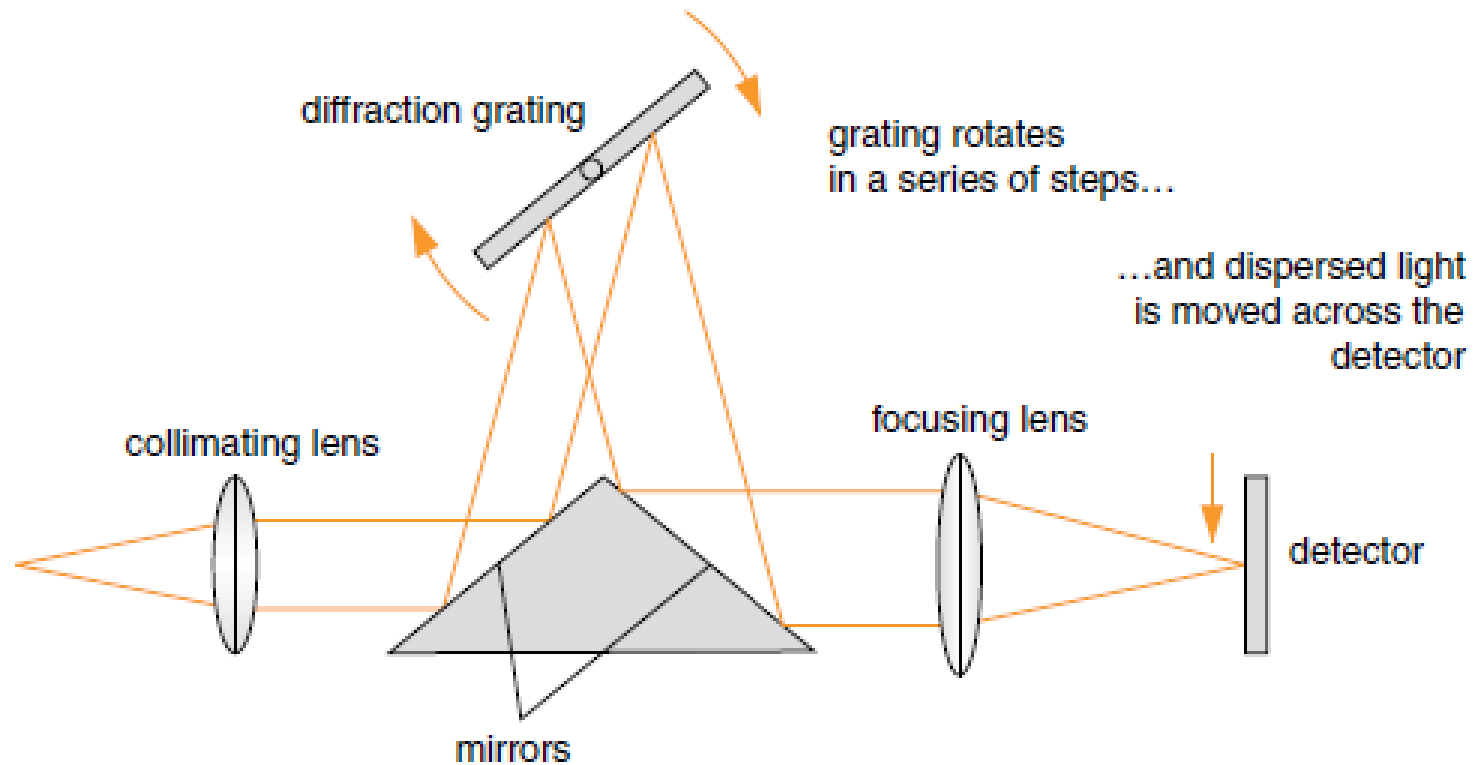
Luminescence
Quenching artefact

SynchroScan



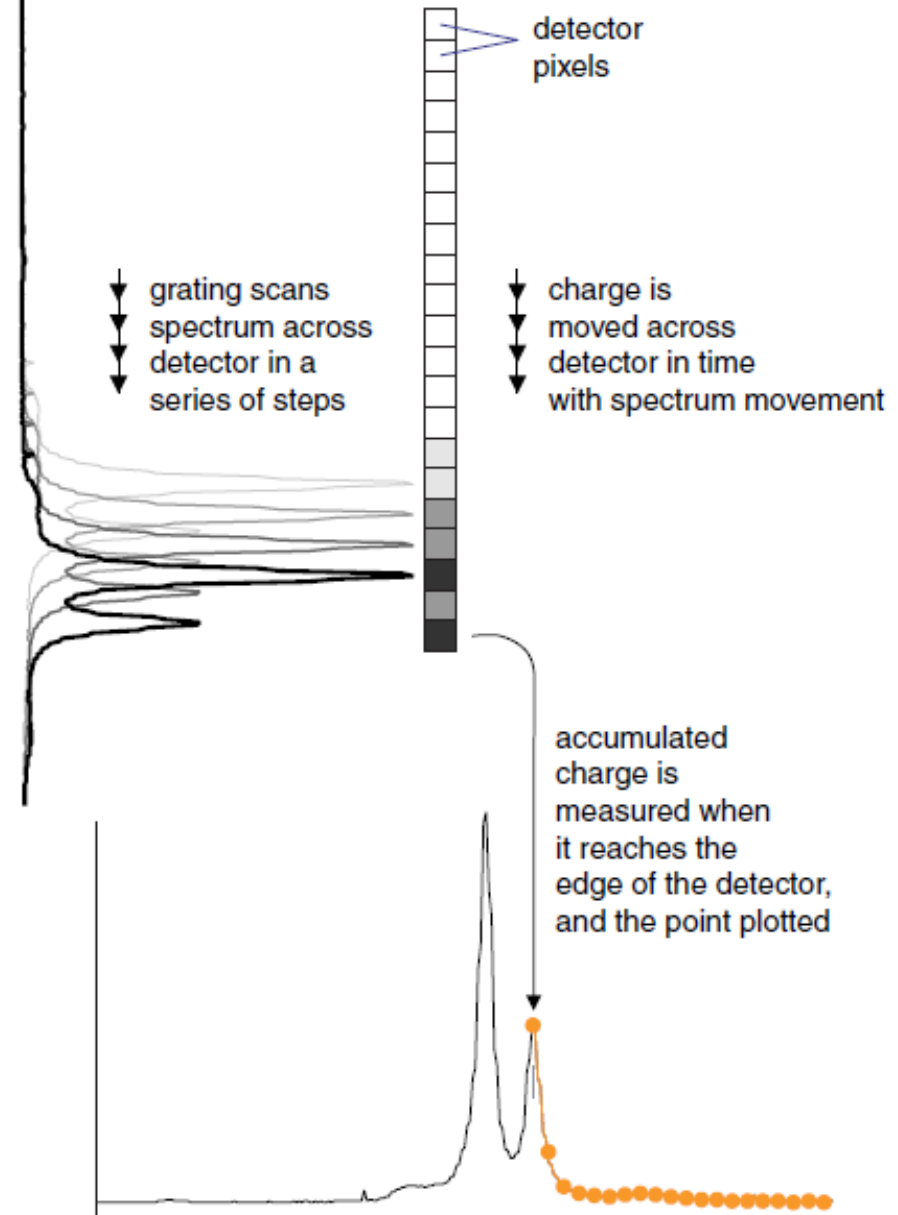
Comparison of SynchroScan
and conventional stitched
spectra

SynchroScan



SynchroScan

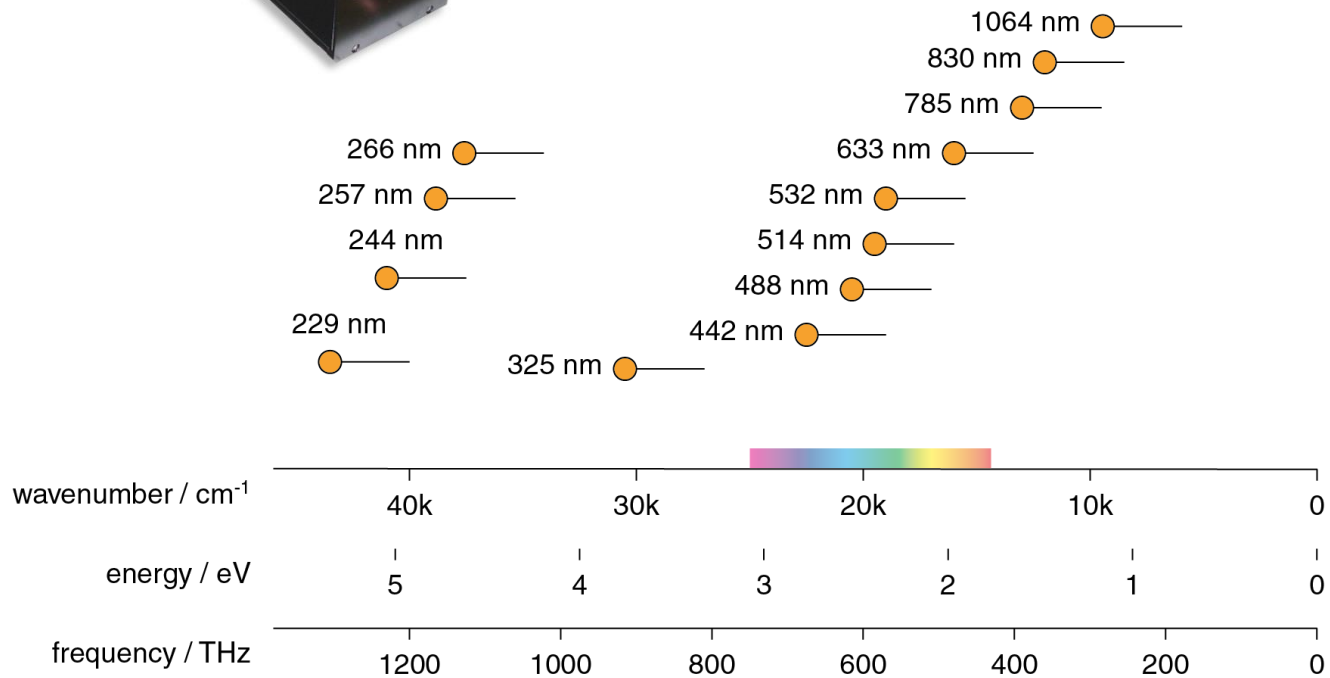
- CCD charge readout synchronized with movement of grating



Laser options



- Lasers available from DUV to NIR



Flexible design for multiple lasers



785 nm NIR
diode laser

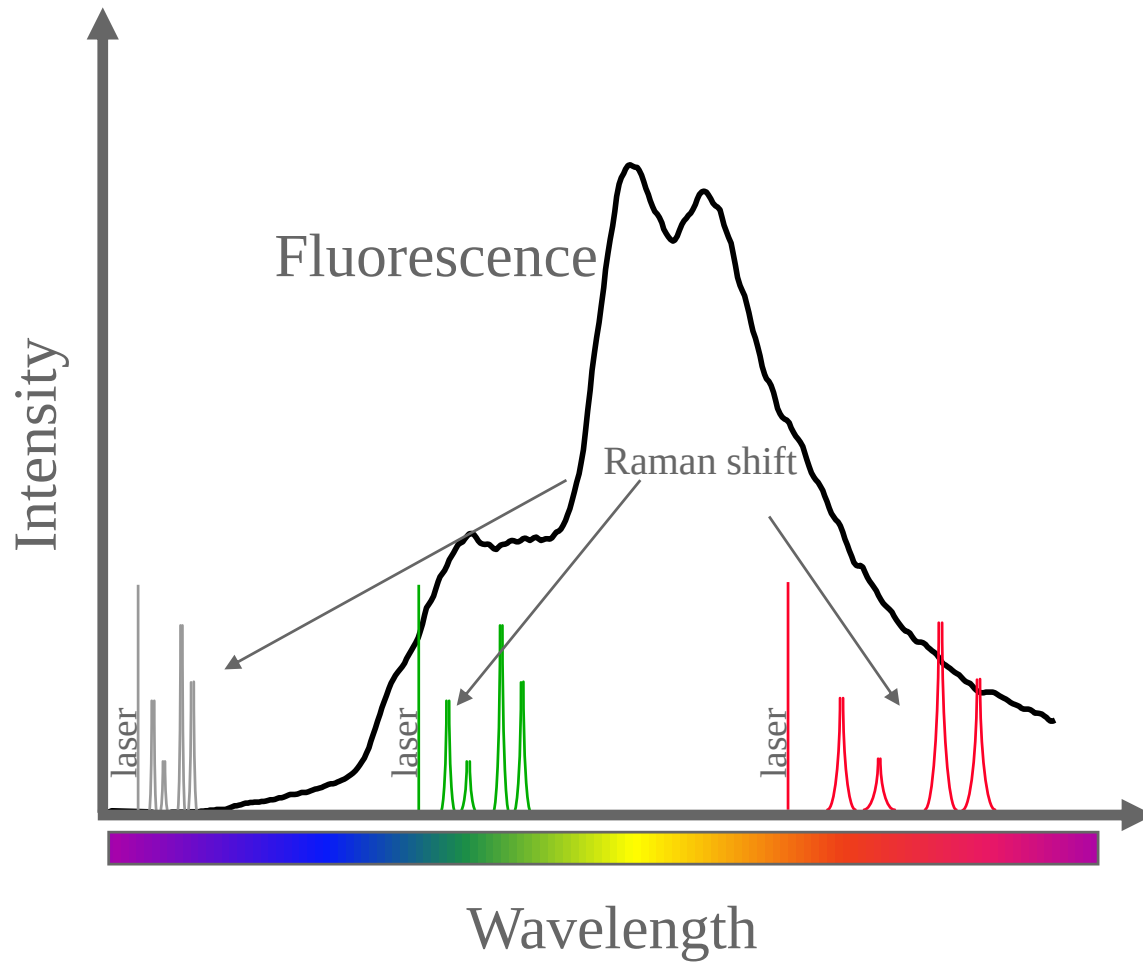
514 nm and
488 nm Ar-ion
laser

633 nm HeNe
laser

325 nm HeCd
UV laser

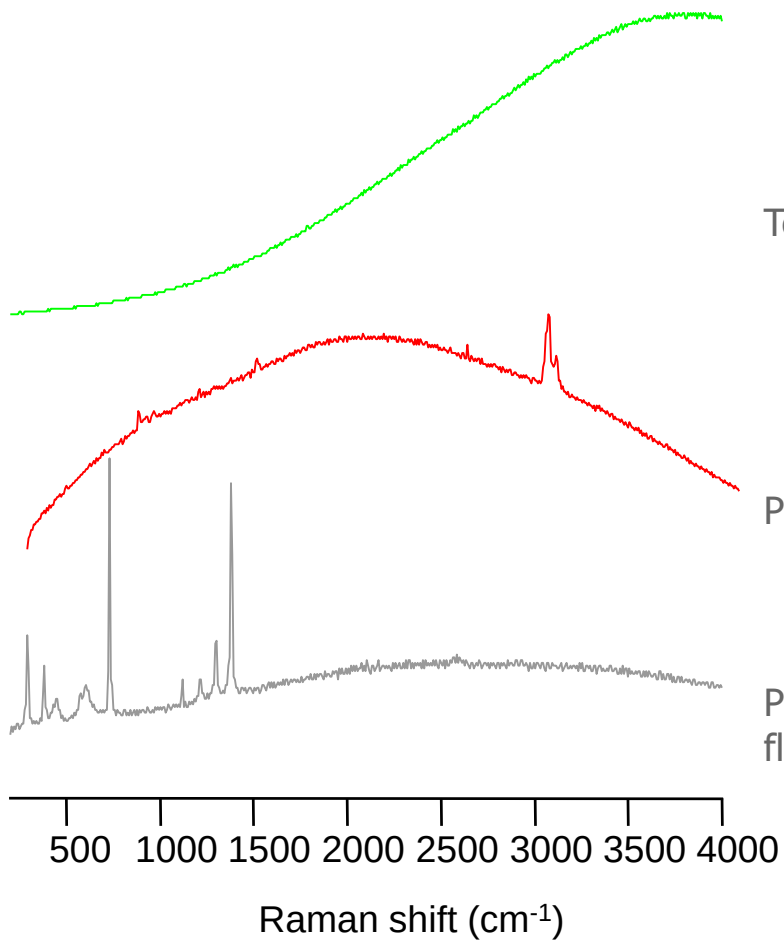
4 Lasers and 5 wavelengths

The problem of fluorescence

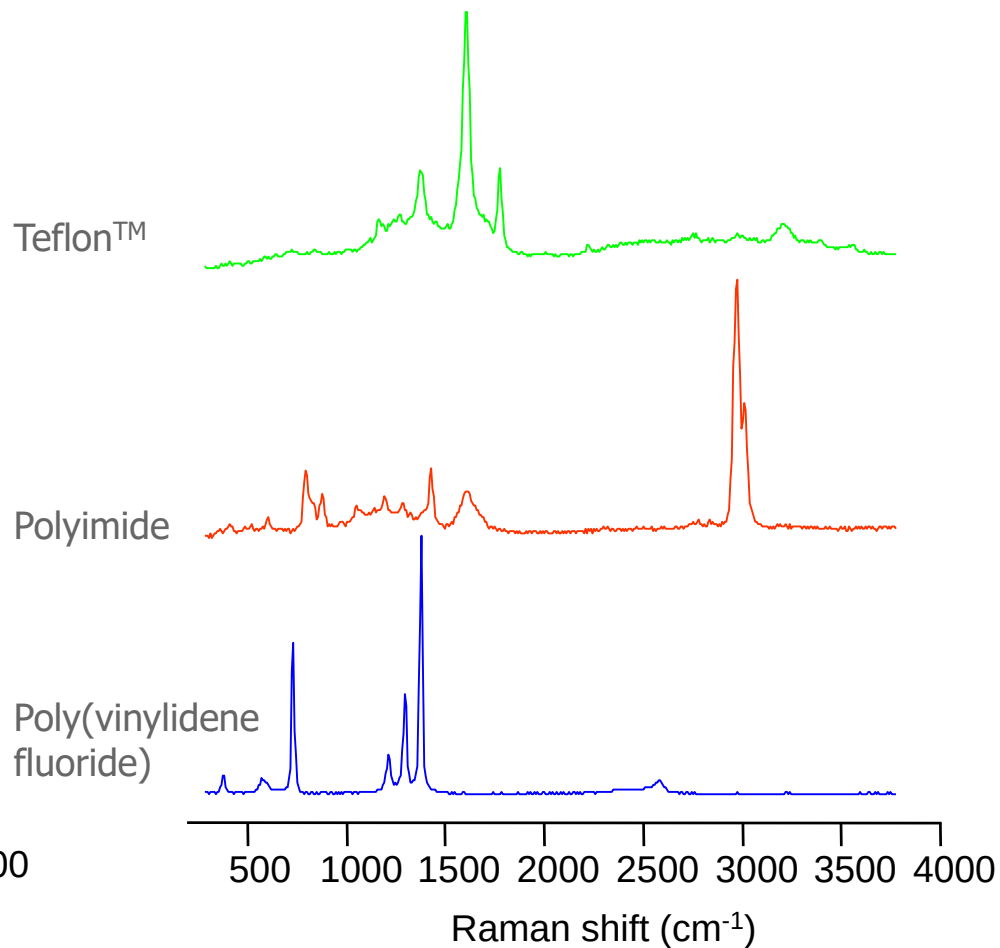


The problem of fluorescence

514 nm excitation

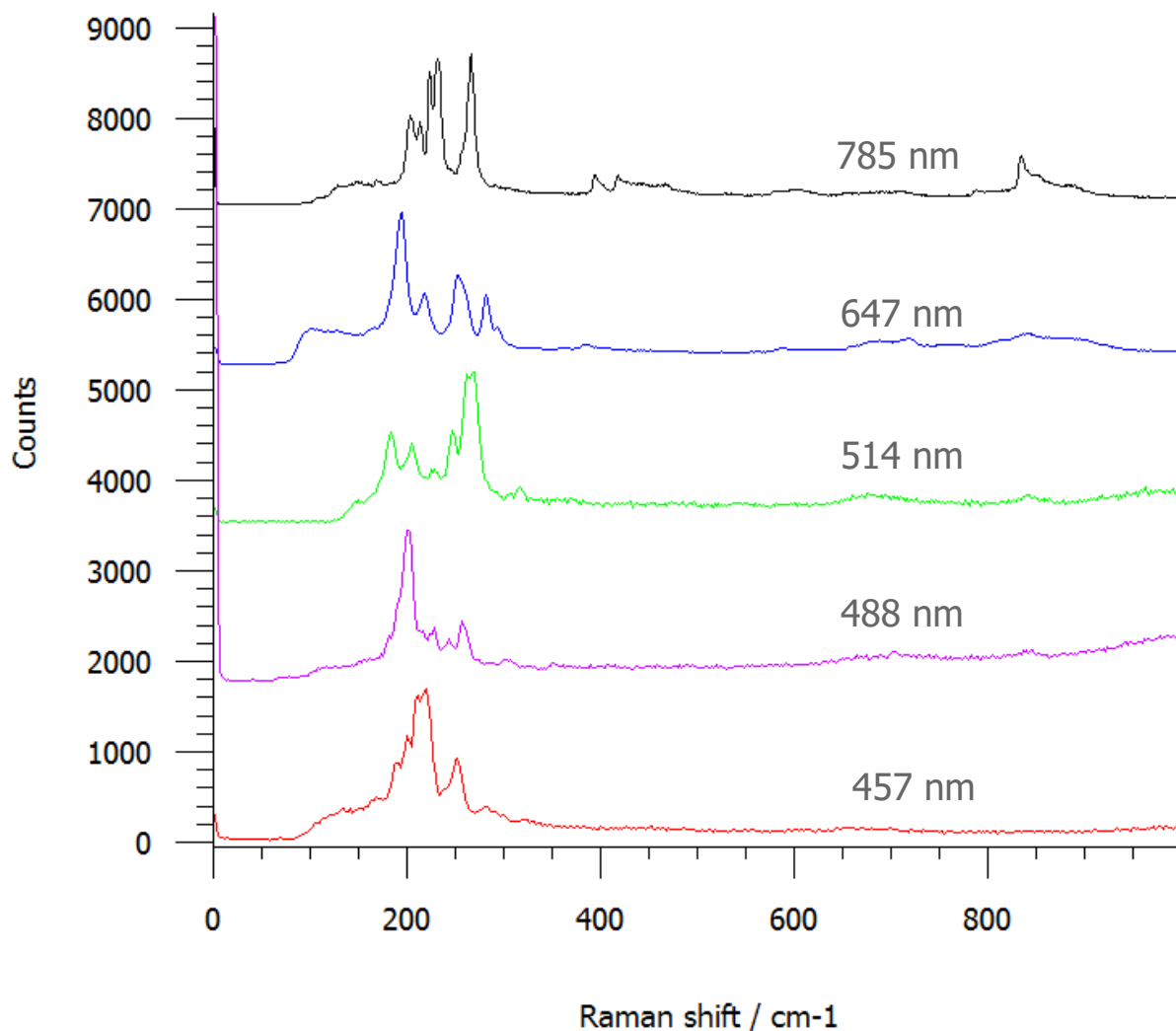


244 nm excitation



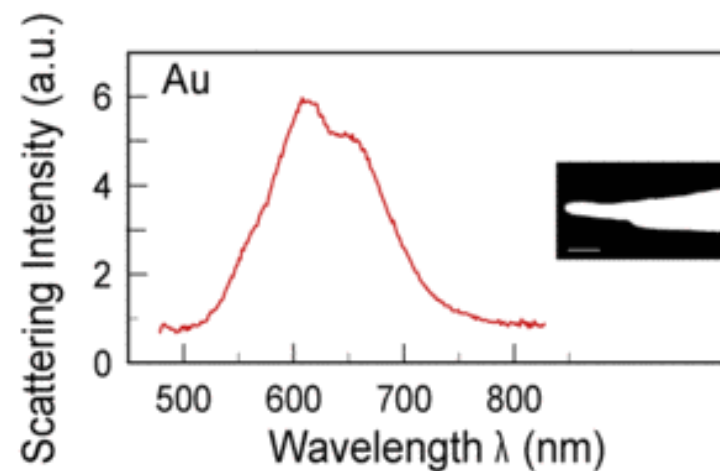
Laser benefits – Resonance Raman

- Resonance Raman occurs when the laser energy is close to an electronic transition in a material and can be 1000 times more intense than regular Raman
- Here resonance Raman is used to probe a cluster of carbon nanotubes
- As the electronic properties of carbon nanotubes are linked to their diameter, it is possible to probe different diameter nanotubes using different laser excitation energies



Laser benefits - TERS

- Tip enhanced Raman spectroscopy (TERS) requires laser excitation source to be resonant with the plasmonic structure of the AFM tip to provide maximum enhancement
- Gold tips – 633 nm laser
- Silver tips – 514 nm , 532 nm laser

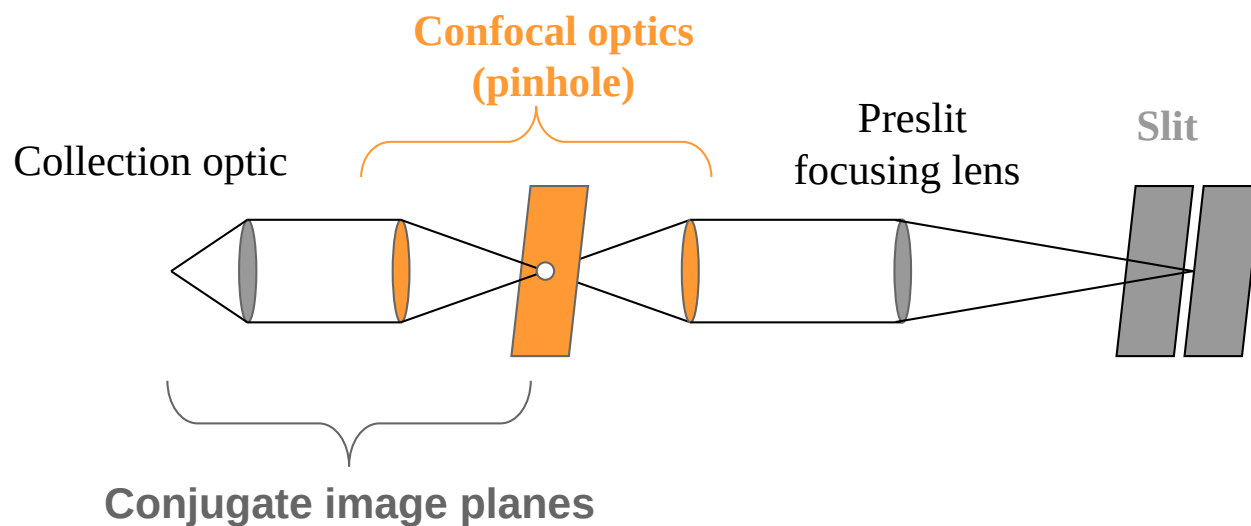


Surface plasmon polariton response of a Au scanning probe tip showing its capabilities for resonant light scattering and local-field enhancement in the visible spectral region (nano-optics.colorado.edu)

Confocal Raman collection

Confocality

“The optimum use of the Raman light flux requires that it be transmitted from the sample to the detector via successive apertures of the instrument. ... The lens coupling system must optically conjugate both the sample(intermediary) image and intermediary apertures with the entrance pupil of the spectrometer.”^a

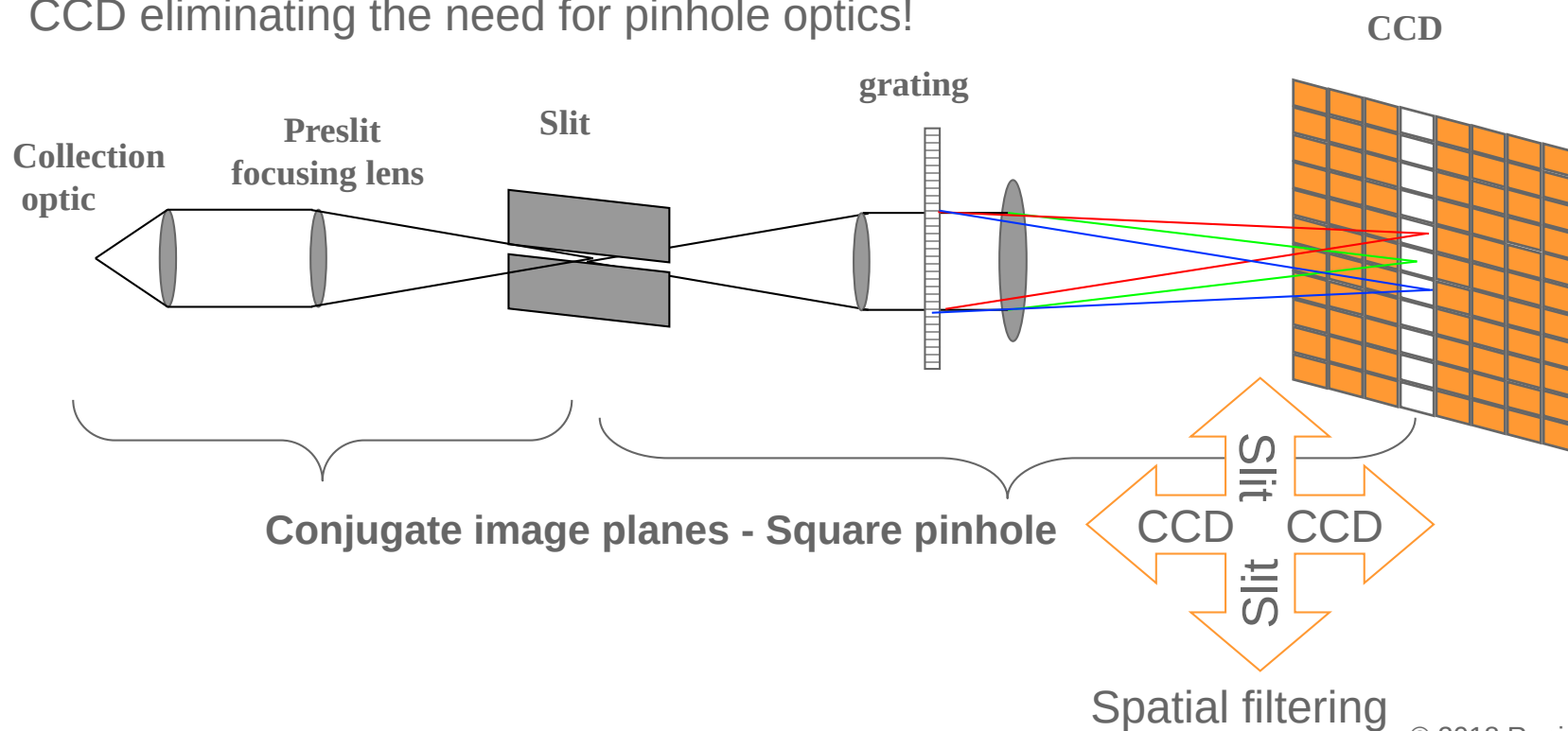


^aG. Turrell *et al.* “Characteristics of Raman Microscopy” in **Raman Microscopy Developments and Applications**, Academic Press 1996.

Confocal Raman collection

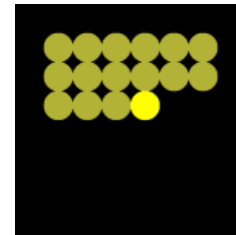
Confocal Raman microscopy without pinhole optics

The use of a stigmatic spectrograph and stigmatic microscope-spectrometer coupling optics creates two additional conjugate image planes at the slit and CCD eliminating the need for pinhole optics!

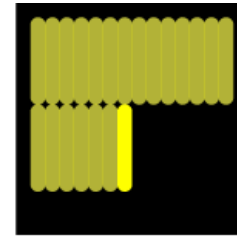


Imaging techniques

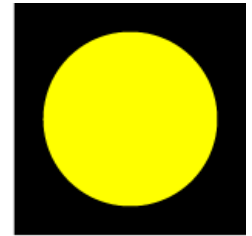
- Raman is a weak effect
 - point mapping speed slow
- Solutions (partial)
 - line focus mapping
 - Raman imaging through filters
 - But for some applications these are still not fast enough



Point
mapping



Line focus
mapping

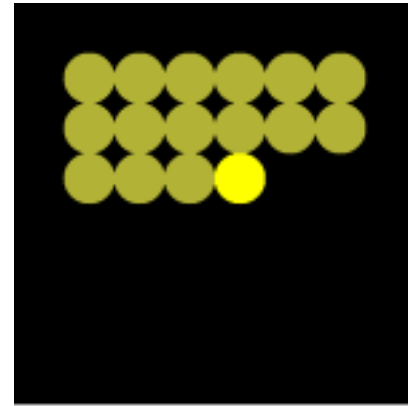


True Raman
imaging

→
faster

Spectral imaging

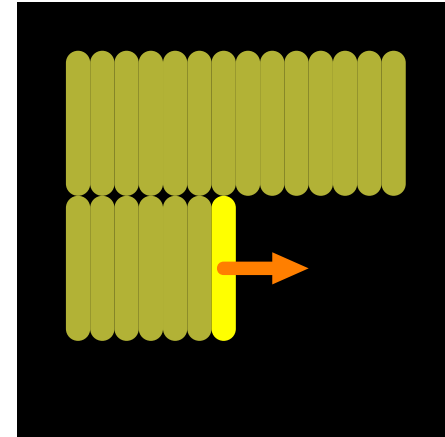
- Acquire data from different points on the sample.
- Generate maps based on parameters of resulting spectra. Examples:
 - Univariate: intensity of band
 - Multivariate: chemometrics:
 - Component analysis based on reference samples
 - Principal component analysis (no references)



Point
mapping

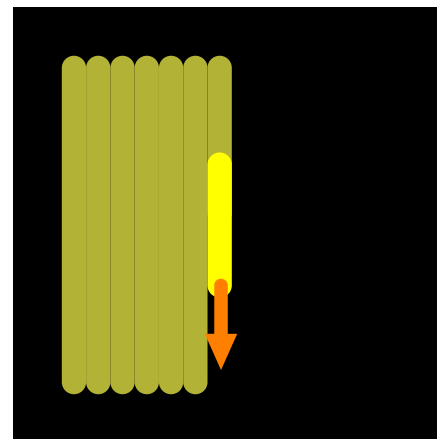
Raman line mapping

- Method
 - Generate laser line on sample
 - Simultaneously acquire spectra from positions along the line
 - Move line over sample, perpendicular to its length
- Advantages
 - Larger area illuminated by line
- Disadvantages
 - Stop/start movement overhead
 - Artefacts...line uniformity



StreamLine™

- Move line the other way!
- Synchronise the stage and the detector
- Advantages
 - Smooth fast continuous movement
 - Artefacts free
 - Power density up to 100× less than point laser configurations
 - Macro and micro (<1 µm) sampling capabilities
 - Zero dead time between sequential spectral acquisitions
 - Multi-wavelength capabilities from the UV to NIR
 - High speed with unparalleled data quality
 - Complete sample coverage with slalom



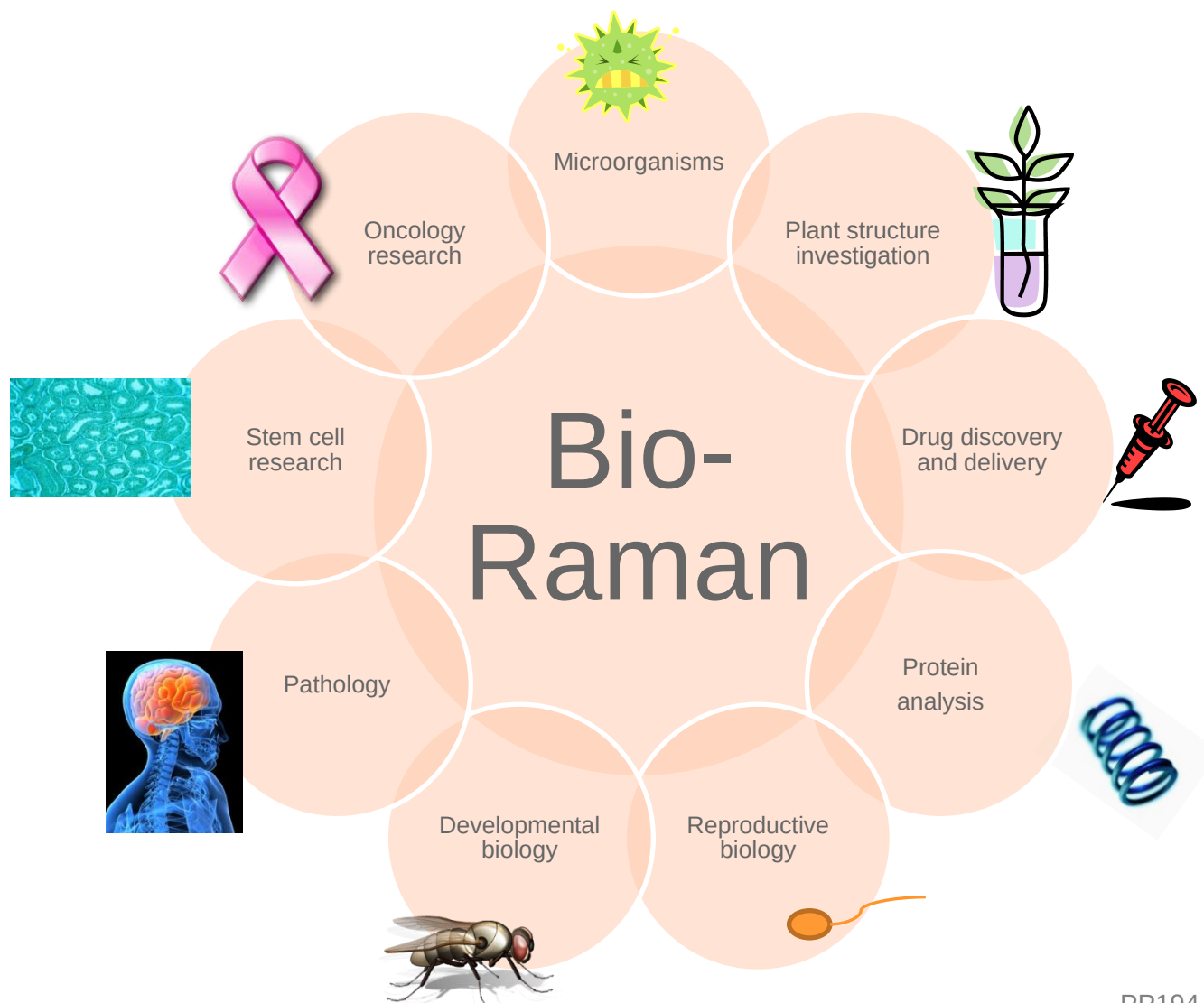
Raman spectroscopy

What are the benefits of applying Raman spectroscopy to bio-studies?

Key benefits of Raman versus conventional techniques

Raman spectroscopy and imaging	Conventional techniques
Label free	Labelling required, may induce artefacts
Provides the full range of chemical info in the spectrum, reveals information on multiple components simultaneously	Requires prior knowledge in order to target molecules, reveals information only on selected molecules
Non-contact and non-destructive • can analyse living cells • downstream analysis of samples enabled • retains spatial information • high resolution (<μm)	Fixation – stops the dynamic process Homogenization – loss of spatial information
Minimal preparation required	Laborious preparation procedures
Water has weak Raman scattering • enables live cell analysis • solution can be directly analysed	Lyophilisation is required for some processes
Measurement condition is easy to optimise	Protocol optimisation required

Examples of Raman spectroscopy bio applications



Raman spectroscopy techniques for bio-applications

Point spectra, micro mapping & time series

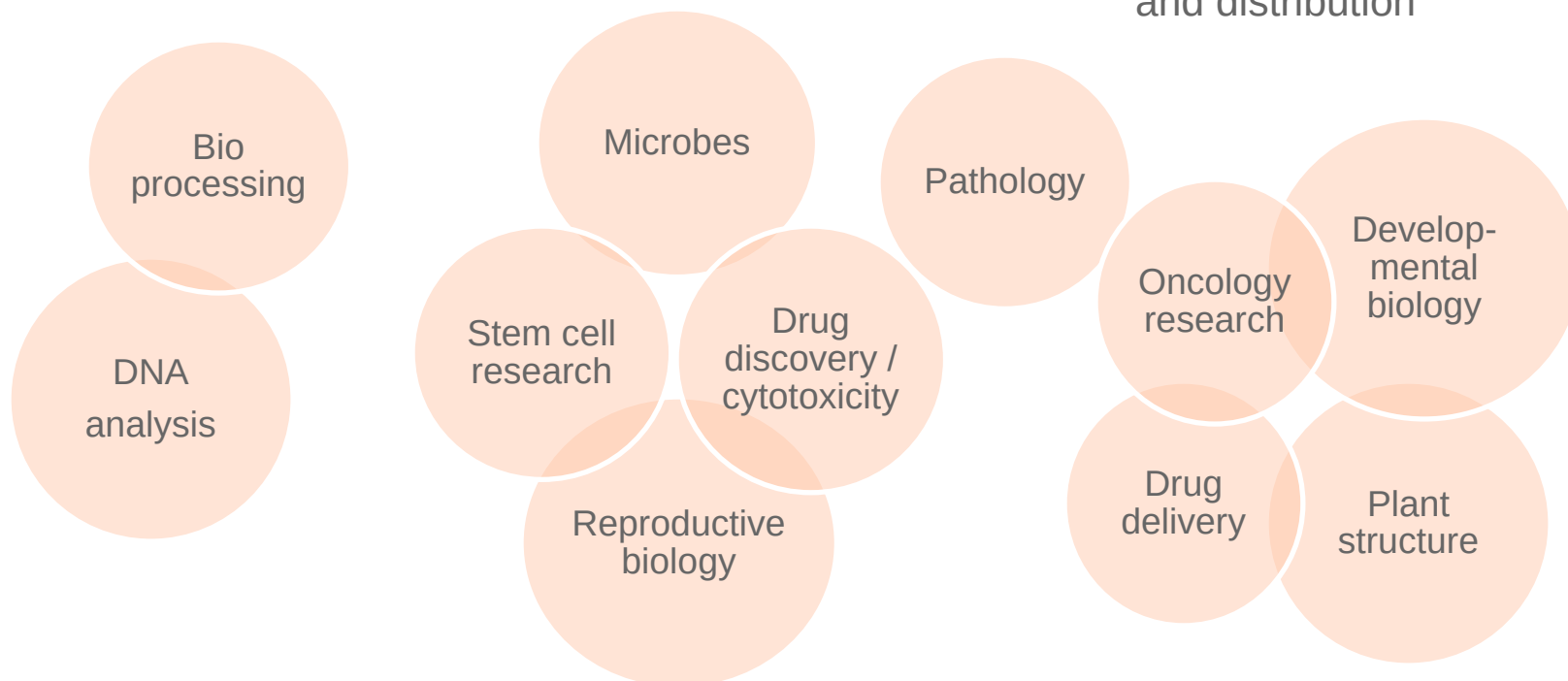
- Analyse molecular / structural changes
- Detect secondary (2°) products

Point spectra and imaging

- Identify cell species
- Monitor cell differentiation
- Examine the cell's health
- Determine disease state

Imaging

- Detect and locate by imaging
- Define anatomical layers and margins
- Monitor changes in size and distribution

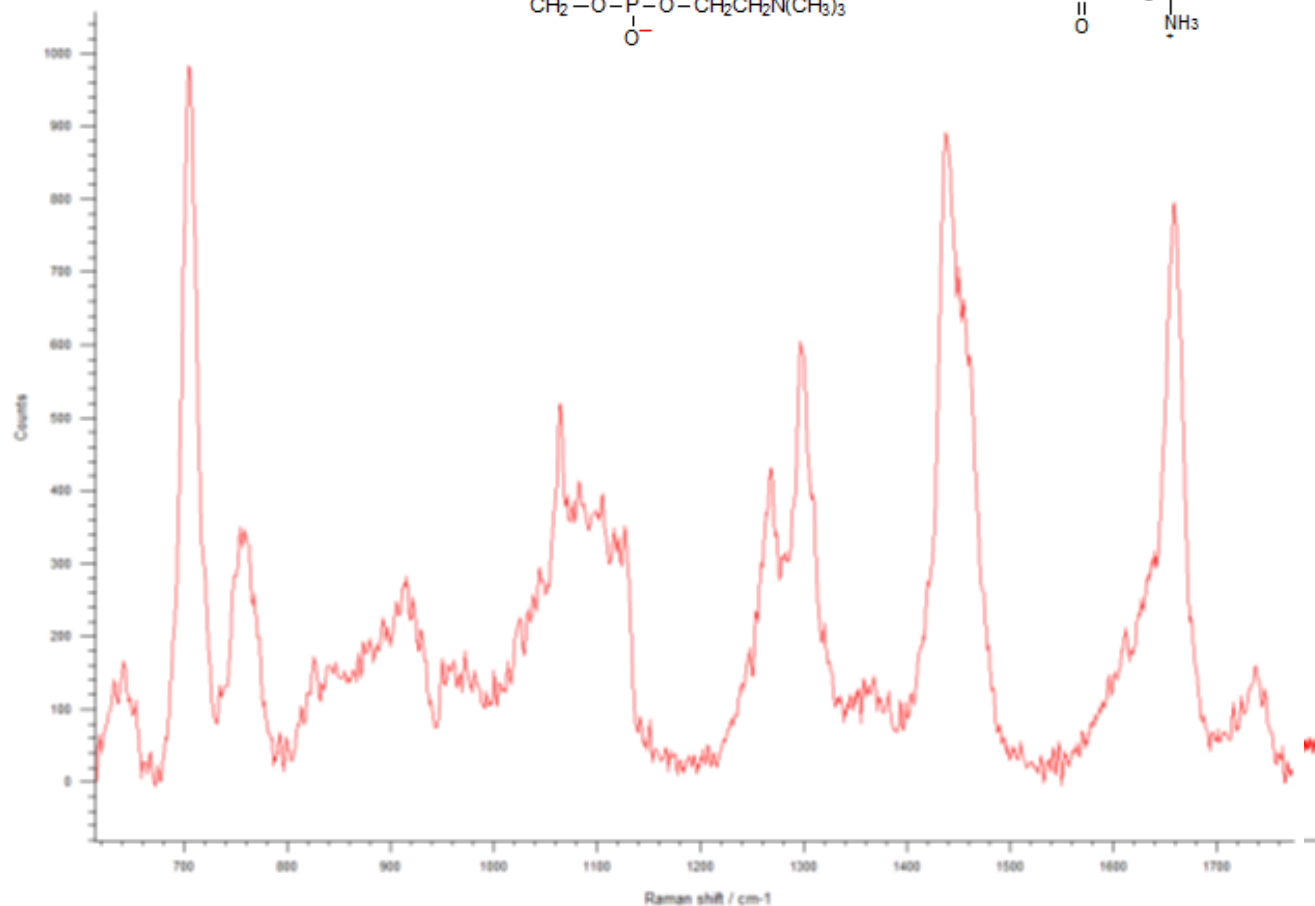
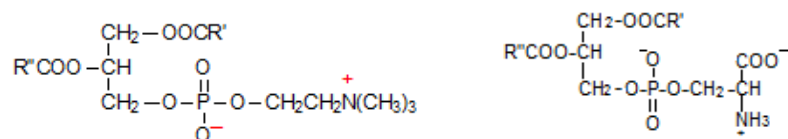


Understanding a bio Raman spectrum

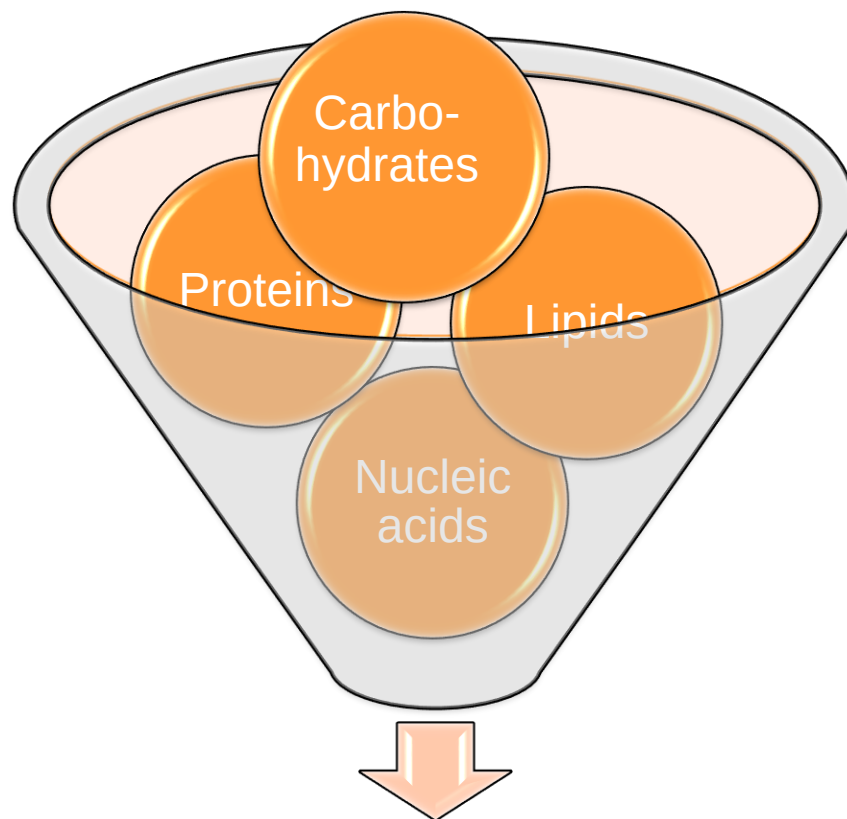
How do we interpret
Raman spectra?

Understanding a bio-Raman spectrum

Phosphatidylcholine + phosphatidylserine (80:20)



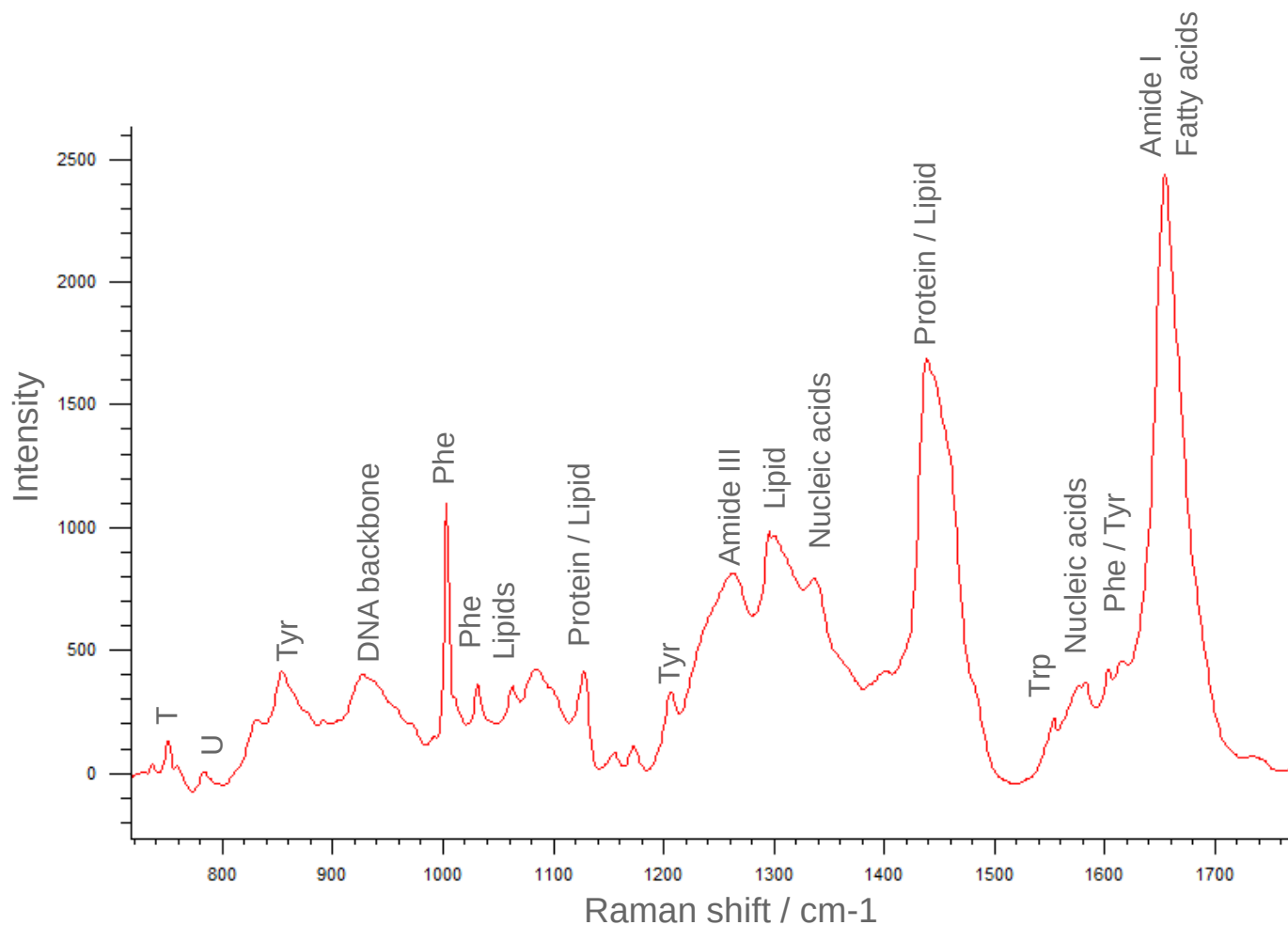
Understanding a bio-Raman spectrum



Raman spectrum captures the sum of all Raman-active cell components and characterises the metabolic cellular status

Understanding a bio-Raman spectrum

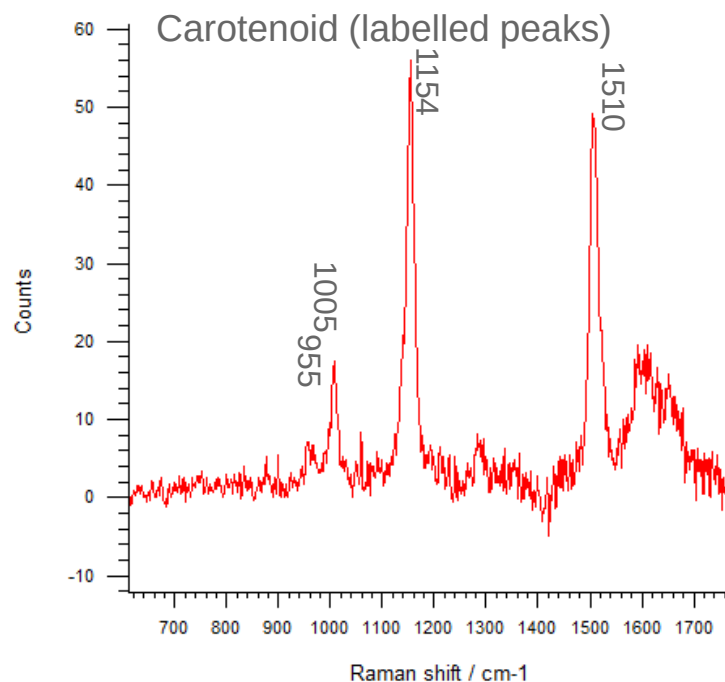
A representative spectrum of H14 S9 human embryonic stem cells



Spectral analysis in bio-applications

- Use of databases for band assignments (e.g. Movasaghi *et al* 2007 Appl Spectrosc Rev 42: 493-541)
- Build up libraries – for regularly used samples/species
- Literature – often contains tables of band assignments from which macromolecular family is inferred

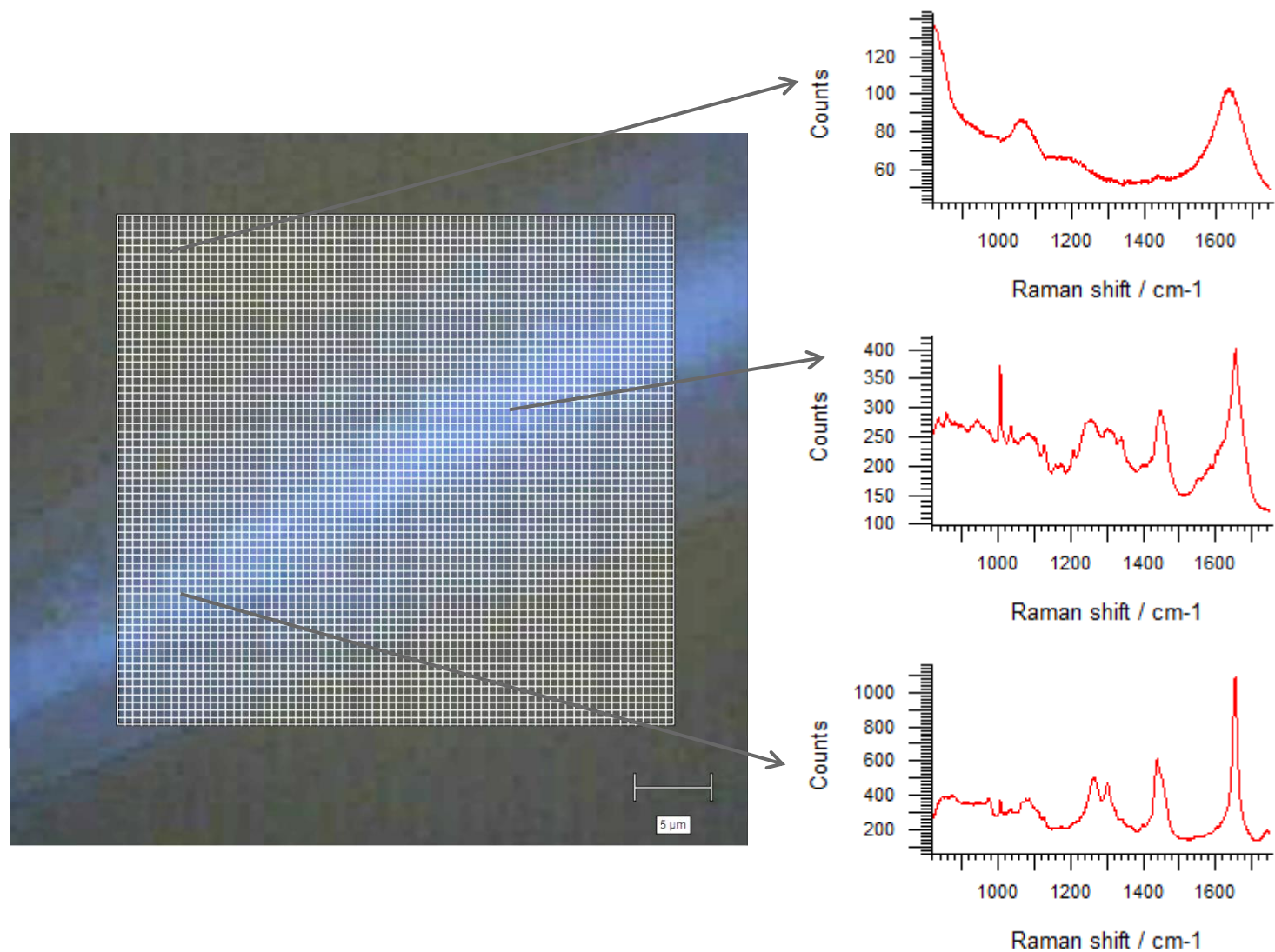
- Rule of thumb: if a macromolecular family is detected, its group of bands should be present
- Single sharp peaks often come from crystalline particles



Moving from point spectra to imaging

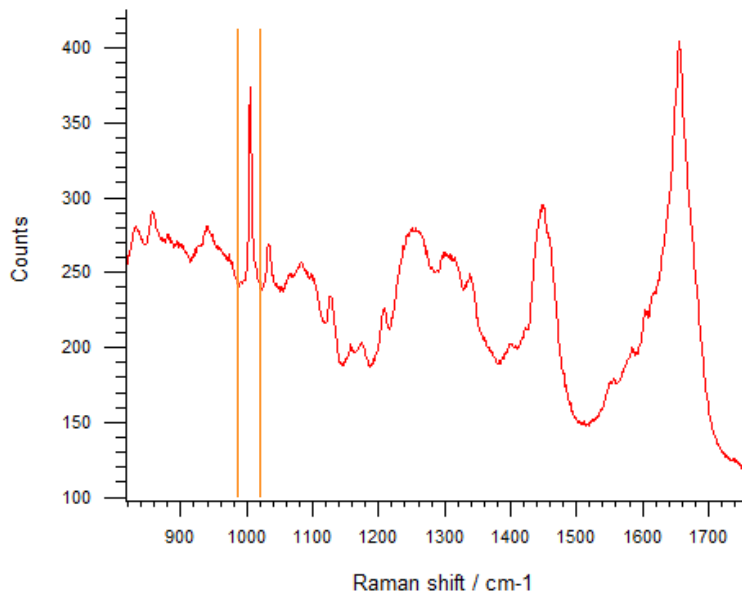
How are Raman images generated?

Generating Raman images

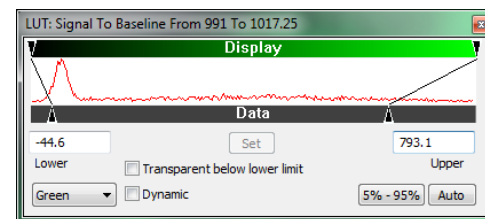
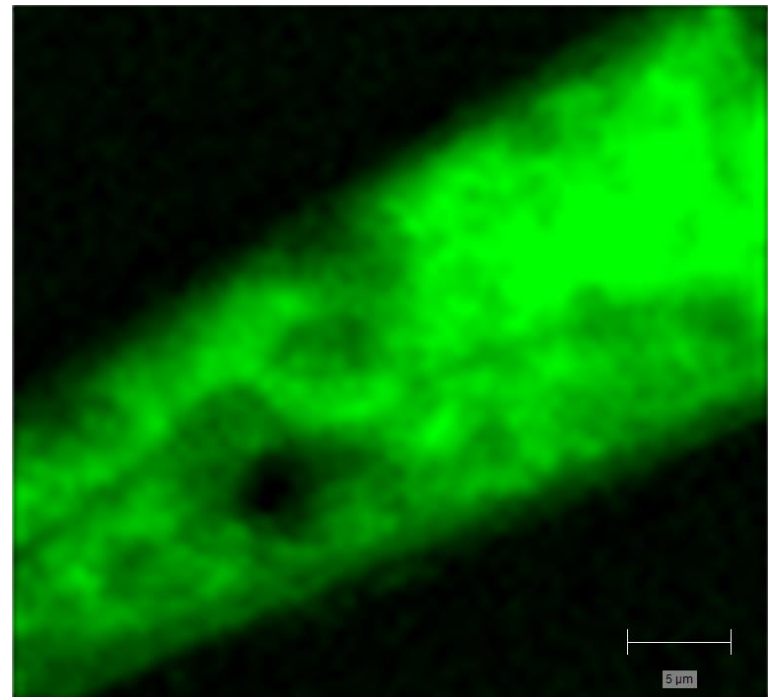


Generating Raman images

Univariate analysis



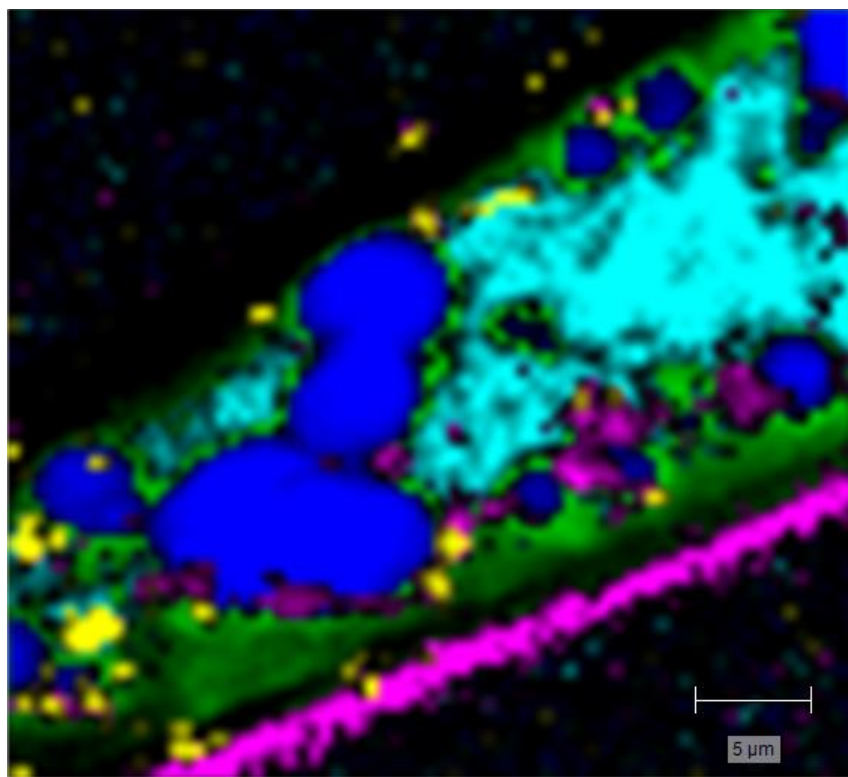
Raman univariate image



Generating Raman images

Multivariate analysis, e.g. PCA
Raman PCA images

PC loadings explain their
characteristics



Application examples

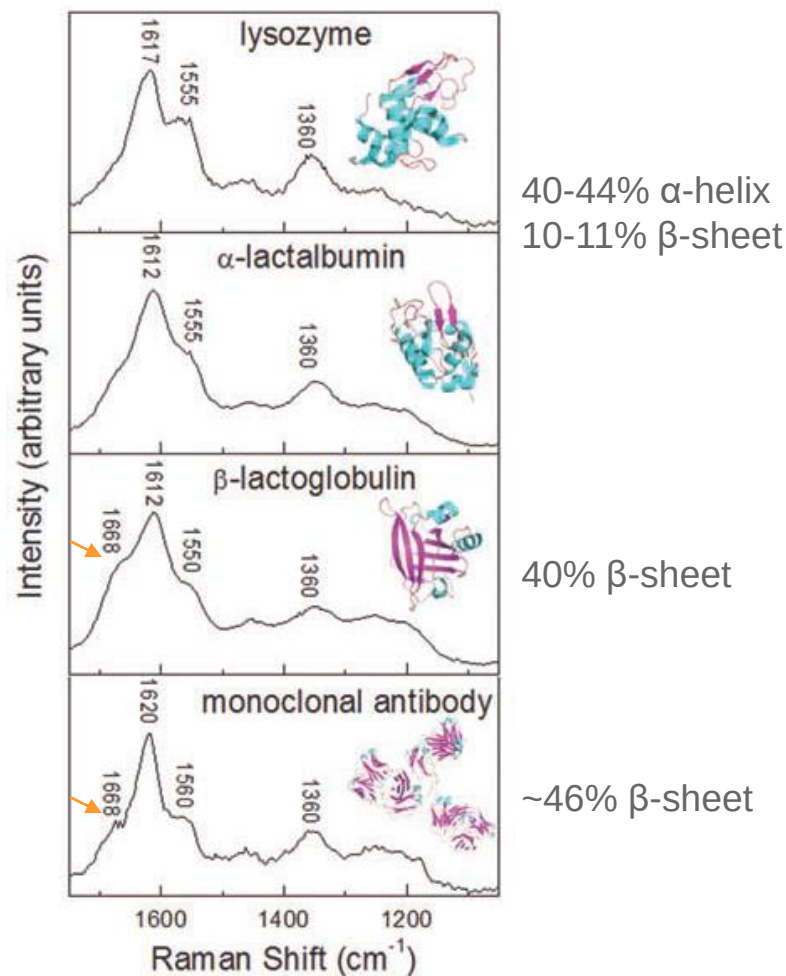
Application examples

Application examples

Application example 1: bioprocessing

Bioprocessing

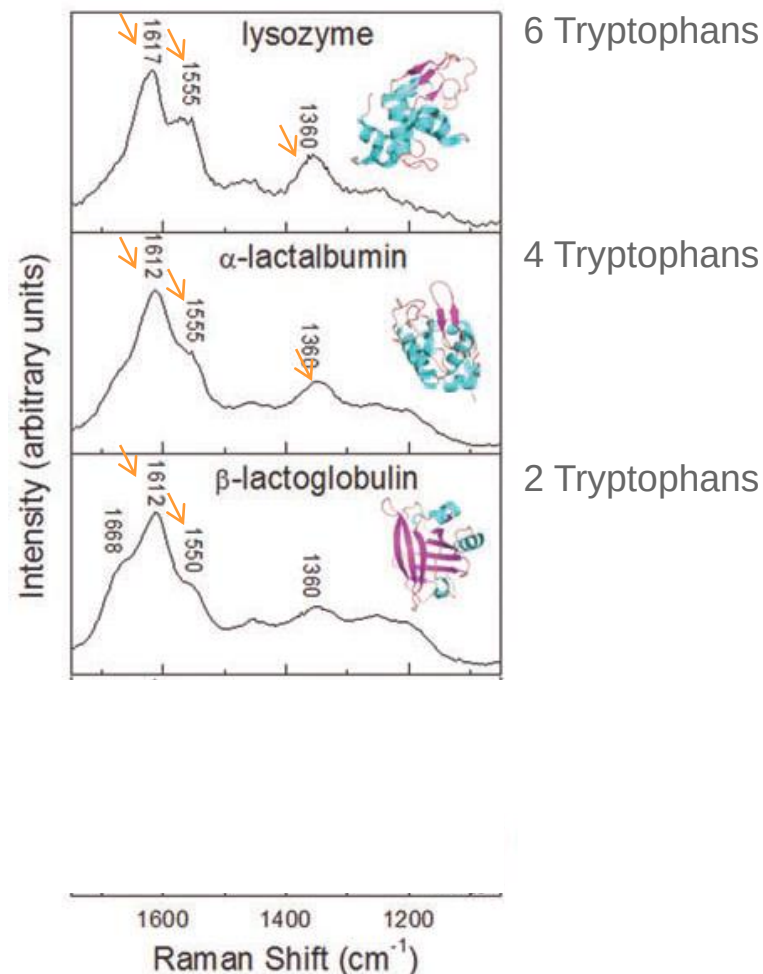
- Monitoring the complex structural changes that occur during protein production
- UV resonance Raman (UVR) is especially sensitive to structural changes, e.g. 2^o structure of protein, aromatic side chains
- DNA, RNA, RNA-protein complex analysis has been demonstrated



Ashton 2011 Eur Pharma Rev 16 (3): 46-49

Bioprocessing

- Monitoring the complex structural changes that occur during protein production
- UV resonance Raman (UVR) is especially sensitive to structural changes, e.g. 2° structure of protein, aromatic side chains, protein unfolding
- DNA, RNA, RNA-protein complex analysis has been demonstrated
- Potential for measuring yields and the presence of 2° products
- 2° products may include glucose, ethanol, secondary metabolites
- On-line and real time monitoring is feasible



Ashton 2011 Eur Pharma Rev 16 (3): 46-49

Application examples

Application example 2:
point analysis for cell and
disease identification

Monitoring DNA integrity in live spermatozoa

- Using sperm with impaired DNA for fertilisation may lead to miscarriage or disorders in children
- Selecting sperm with intact DNA for fertilisation treatments potentially improves the success rate of fertilisation
- Optical trapping of live sperm and Raman analysis of their DNA can determine the healthiness of the sperm



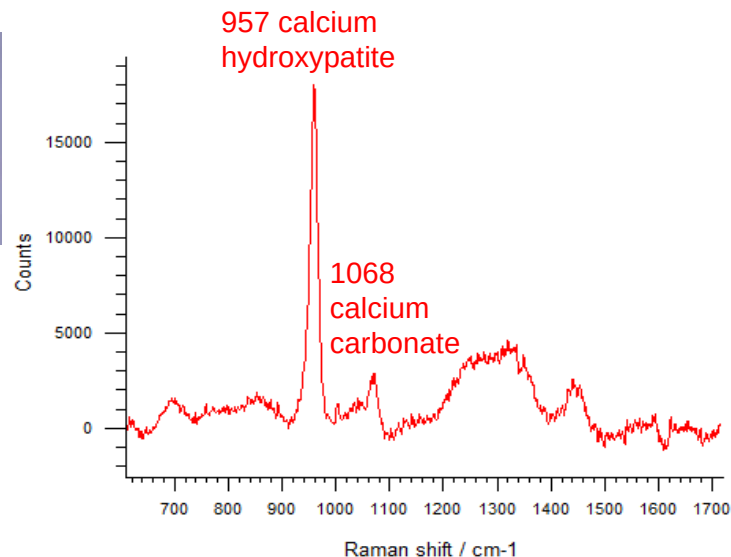
- Dr. Alistair Elfick and Ruby Raheem, University of Edinburgh, UK.
- Dr. Rod Balhorn, Dr. Stuart Meyers and Dr. James Chan, University of California, USA

Assessment of disease state – atherosclerosis example

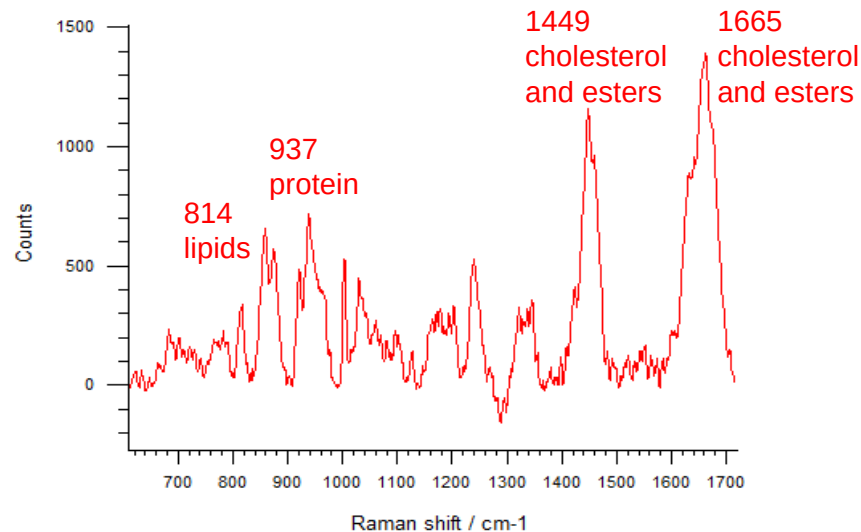
- Atherosclerosis – assessing pathological states
- Calcification or presence of plaques can be shown in the Raman spectra of arteriole lumen



Calcified
carotid artery

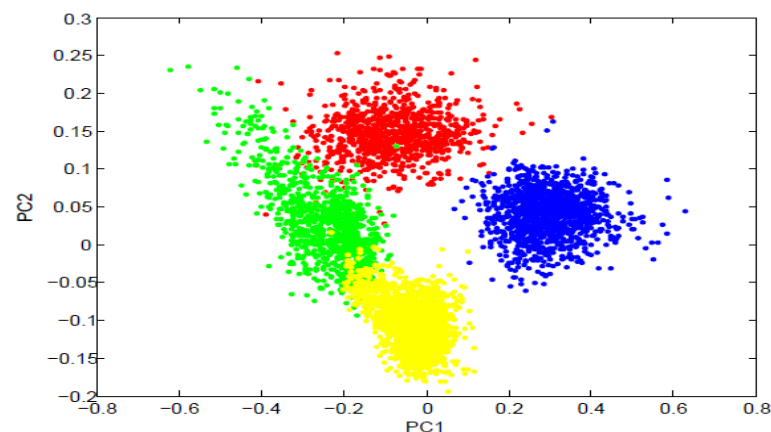
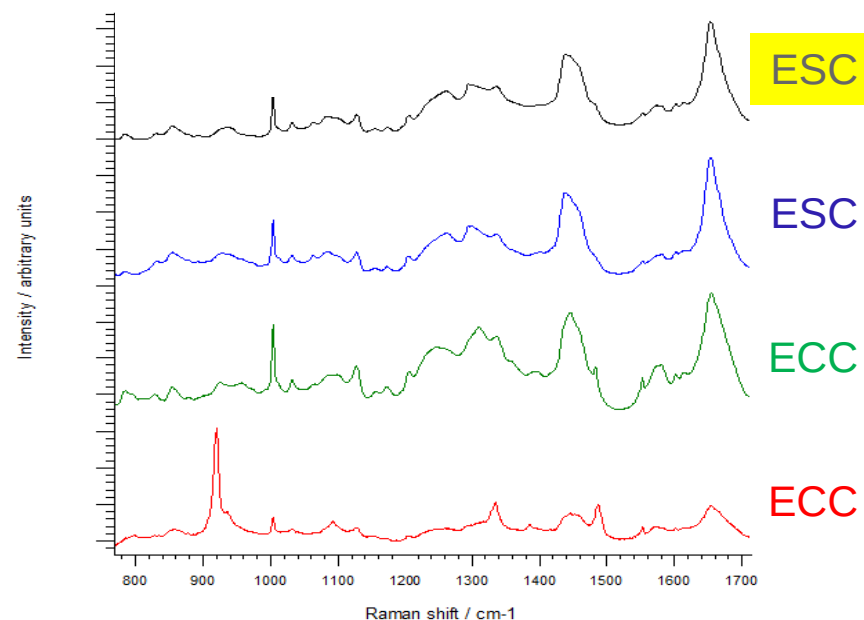


Atherosclerotic
artery



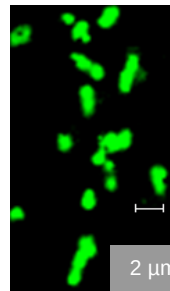
Identification of human embryonic cancer/stem cells

- The status of stem cells and their differentiated cells is at present monitored through their surface antigens, or reporter genes
- Drawback: potential interference with the cells and the dynamics of the substates
- Human embryonic stem cells (hESCs) and human embryonic cancer cells (hECCs) are identified here based purely on their Raman spectra, via chemometric analysis
- Their chemical differences are explained by the Raman bands

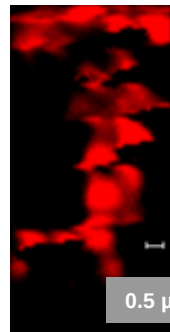


Identification of bacterial strains

- Bacteria can be identified by their Raman features
- Raman spectroscopy of microorganisms enables rapid diagnosis of bacterial infection
- StreamLineHR imaging resolves their fine structural details
- Imaging also enables 1000's of spectra to be collected in an automated fashion, resulting in more representative reference spectra



Amide I intensity
distribution of
E. coli



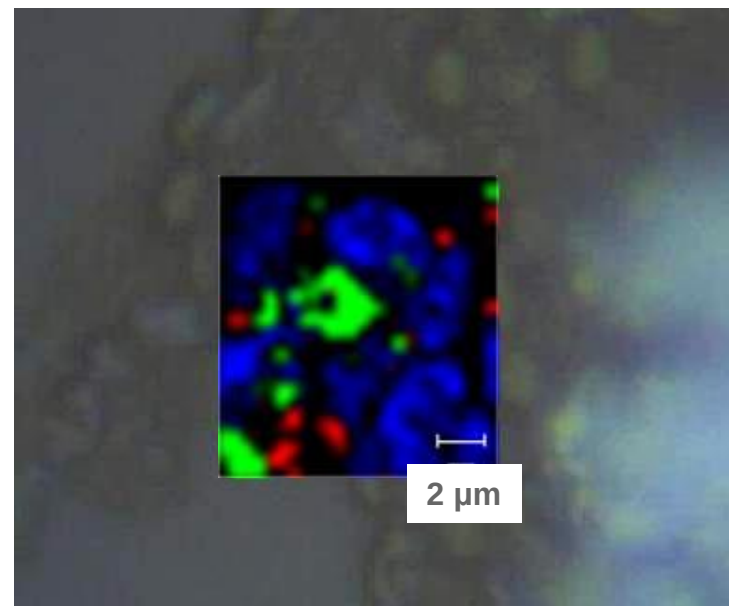
Amide I
intensity
distribution of
S. epidermidis



Samples courtesy of IPHT, Jena, Germany

Identification of microbes in a heterogeneous environment

- A representative Raman fingerprint of each strain was established through StreamLineHR imaging
- Component analysis was applied to identify the species based on their pure reference spectra
- Strains were distinguished even through subtle variations



DCLS component analysis applied to Raman spectra of bacteria and yeast strains.

B. subtilis

S. cerevisiae

S. epidermidis

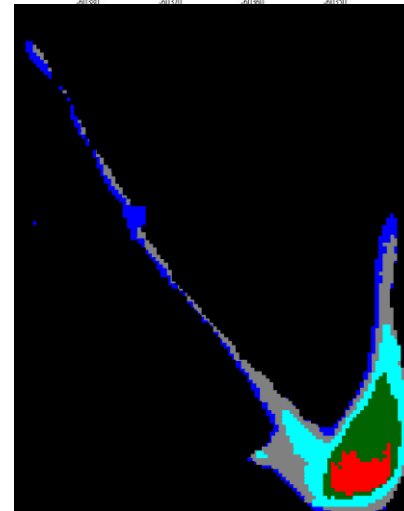
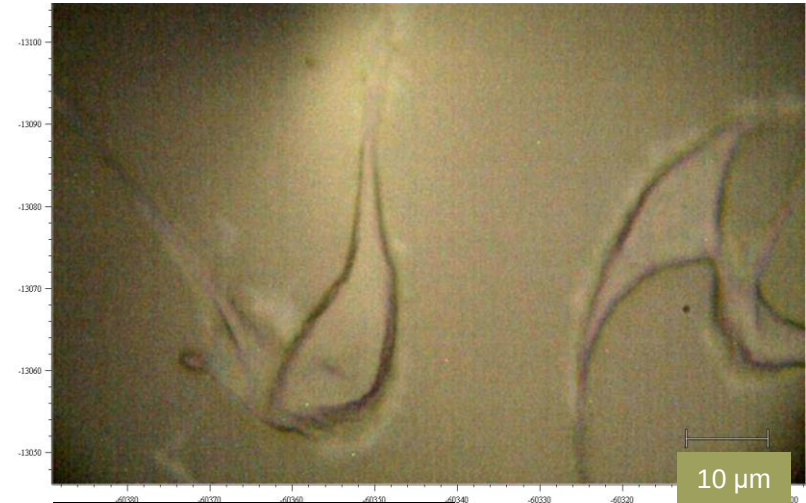
Samples courtesy of Roy Goodacre, University of Manchester, UK

Application examples

Application example 3:
Raman imaging to reveal
distribution of bio species

Cellular components study – 2D glioma cell example

- Monitoring changes in the chemistry, size and distribution of subcellular structure is a crucial part of cell biology and oncology research
- StreamLine imaging and clustering analysis were applied to a glioma cell
- This enabled its subcellular structures at sub-micrometre spatial resolution to be determined

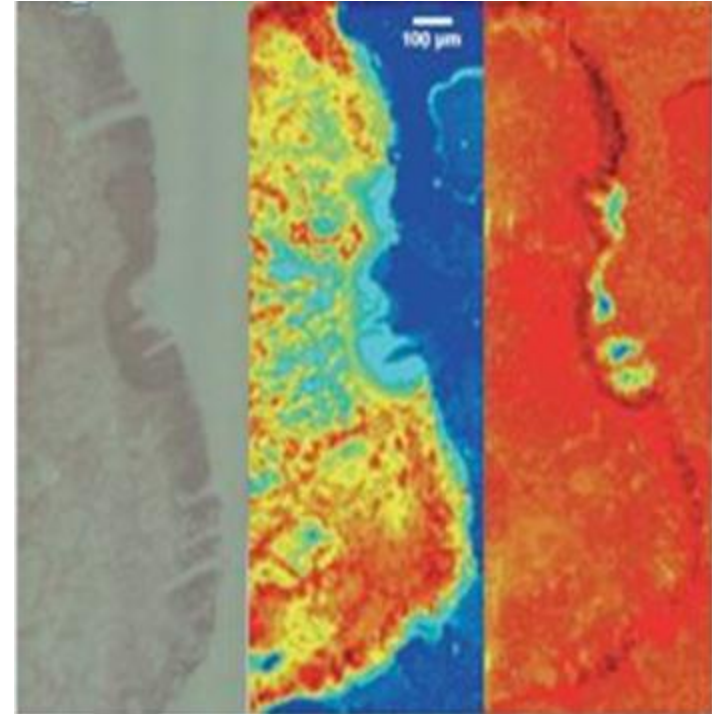


StreamLine image
Hierarchical clustering
analysis (HCA)

Blue, grey – processes
Cyan – cytoplasm
Green, red – nucleus

Tissue imaging esophageal cancer – Barrett's mucosa

- Chemical information from Raman spectra indicates presence of malignant tissue
- StreamLine imaging provides spatial information, and therefore shows the location and boundary of the tumour
- Metastases can be identified
- Trained algorithms can be used to identify cancer stages

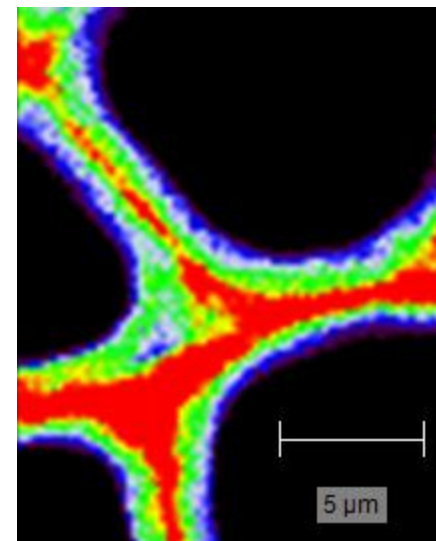
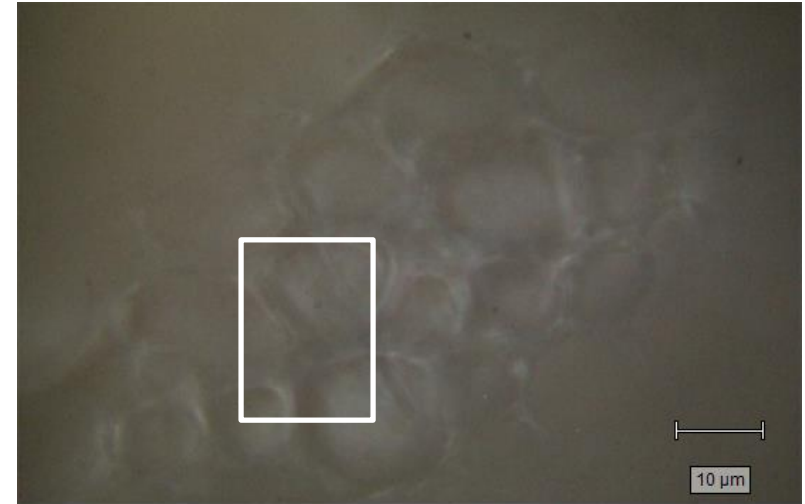


Left: histological section
Centre: PCA identifies muscle tissue
Right: PCA identifies blue regions as normal squamous and dark red regions as Barrett's mucosa

Data from Prof Nicholas Stone, University of Exeter, UK

Maize cell lignin and cellulose imaging

- Imaging of plant cells enables lignin and cellulose distribution to be visualised and quantified *in situ*
- This application is important in agricultural, material, and bio energy research
- Excellent spatial resolution
- Enabled the thin lignin layer to be resolved

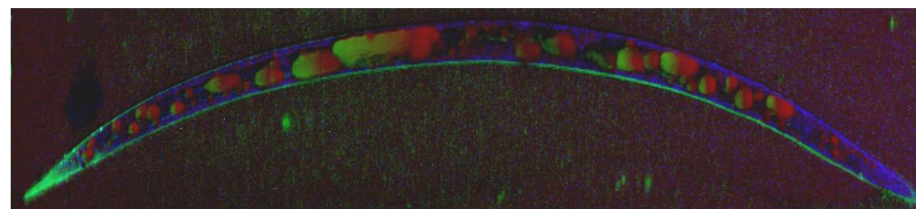


Maize root
embedded in resin
and sectioned

Lignin
Cellulose

Raman imaging of a whole model organism

- Nematodes, zebrafish, Drosophila etc. are model organisms for human diseases
- Assessing the phenotypic changes and observing changes in patterns is important for research using model organisms
- Staining may lead to the generation of artefacts
- StreamLine imaging enabled rapid and high resolution imaging of the entire organism within a few hours.
- Domains with different chemical abundance resolved by principal component analysis (PCA)



Steinerema kraussei

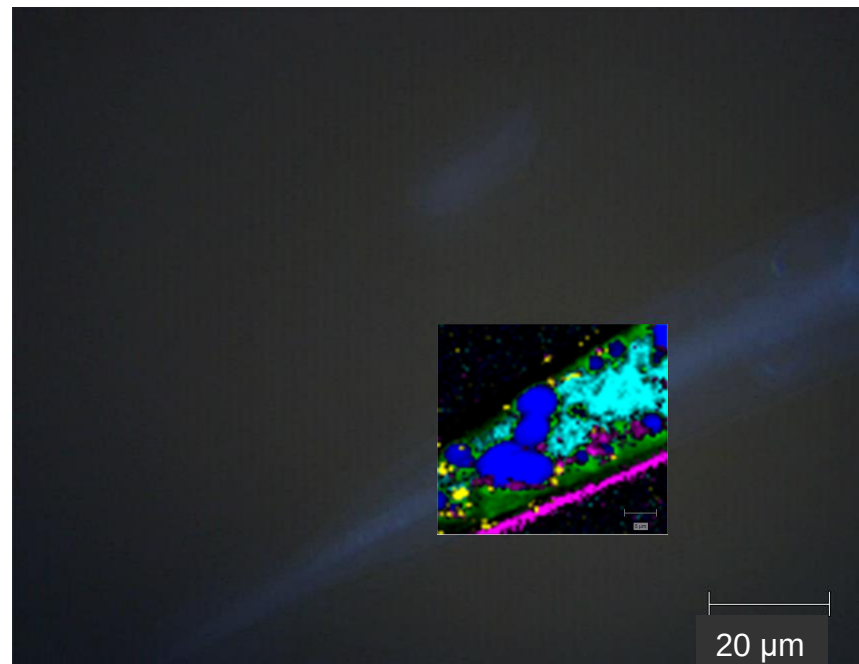
Lipid domains

Collagenous cuticle

Protein rich cellular layer

Raman imaging of a model organism

- StreamLineHR imaging (step size 500 nm) enabled fine details in the selected area to be resolved



Green – protein and lipid
Cyan – muscle layer
Magenta – collagenous cuticle
Blue – lipid rich domains
Yellow – globins

Why CARS? Basics

- Biggest problem for Raman: fluorescence
- Fluorescence has fixed wavelength
- Fluorescence usually is red shifted (i.e. towards lower energy)
- Raman has fixed energy shift:
 - Stokes – red shifted
 - Antistokes – blue shifted
- One way to get rid of Fluorescence: choose a suitable excitation wavelength.
- Another way: use anti stokes because it is blue shifted!
- BUT: Anti Stokes is due to quantum mechanics much weaker than Stokes!

Basics of CARS

Solution: CARS

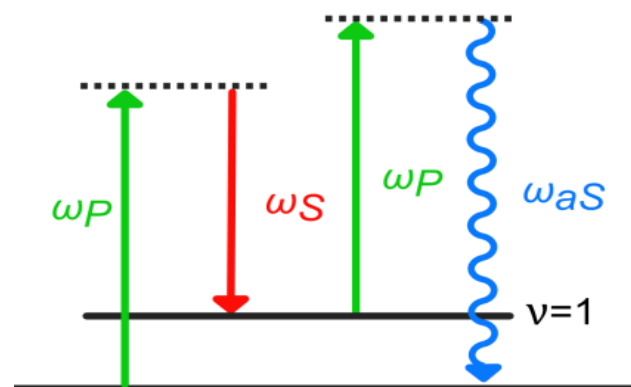
Non linear 4-wave-mix process

Pump laser with frequency ω_P

Stokeslaser with frequency ω_S

CARS- frequency $\omega_{\text{CARS}} = 2\omega_P - \omega_S$

CARS = coherent
Anti-Stokes Raman
Scattering



Basics of CARS

Advantages:

CARS is NOT spontaneous Raman process

Coherent stimulated scattering process

Therefore the CARS signal is coherent, too

BUT: the signal has due to the coherent characteristic preferred direction

→ CARS gives best results in transmission

Theory of Raman – Quantum mechanics (fully animated) *

Rayleigh, Stokes, Anti-Stokes and a little bit of quantum mechanics

- **Case 2: Stokes Scattering**

→ Stokes Raman scattering is caused by an incident photon exciting a vibrational state and then relaxing to the ground state. The light that is emitted has a lower energy than the excitation wavelength.

→ it doesn't matter whether it starts from ground state and goes back to this or an already excited state and relaxes back to this one.

